Silkworm egg production
Volume III

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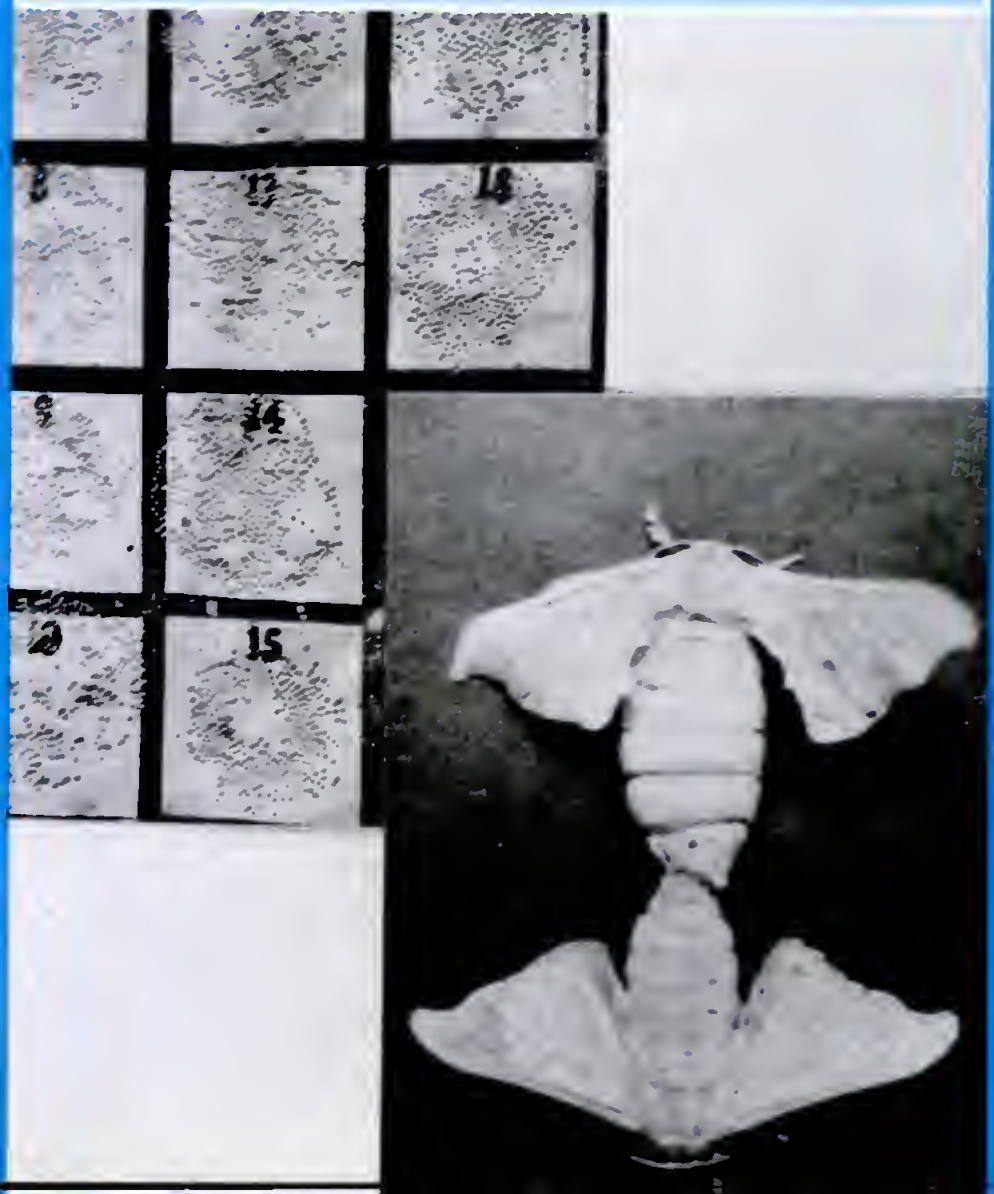

FOOD
AND
AGRICULTURE
ORGANIZATION
OF THE
UNITED NATIONS

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Written by
Wang San-ming
Translated by
Li Ping-yi, Pan Run-shi
and **Ou Bing-sen**

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FOREWORD

Silk, "the queen of fibres", is admired by people the world over, and silk and silk products are always in great demand. Endowed with favourable conditions for mulberry cultivation and silkworm rearing, the Asia-Pacific Region has great potential for sericulture; many developing countries in this region are eager to tap this potential. In order to meet their needs and promote development of sericulture in the region, the Government of the People's Republic of China and the Food and Agriculture Organization and the United Nations Development Programme (FAO/UNDP) have jointly established the Regional Sericulture Training Centre for the Asia-Pacific Region (RSTC) in the South China Agricultural College, Guangzhou, for training professionals in sericulture in 200-day sessions.

These manuals, which offer an outline of sericultural science and technology, have been compiled as teaching materials for RSTC participants. In view of the specific circumstances of the participants, the manuals stress technical expertise for sericultural production in tropical and sub-tropical areas and introduce China's unique experience in silkworm rearing. It is hoped that the manuals will be useful to sericulturists not only in the Asia-Pacific Region but also in other parts of the world.

The authors wish to express their deep appreciation to FAO for making it possible to publish these manuals and to Mr. R. Karam for his invaluable advice. They also wish to thank the Sericulture Research Institute of the Academy of Agricultural Sciences of China and the Sericulture Department of Zhejiang Agricultural University for their valuable comments on the manuscripts.

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CHAPTER 1

INCUBATION OF PARENT SILKWORM EGGS

1.1 Purpose of Incubation

The main purpose of incubating parent silkworm eggs is the uniform development of the embryo, and thus uniform hatching achieved through proper maintenance of environmental conditions. It is carried out at predetermined dates when parent eggs have been brought out of diapause or artificially hatched according to the physiological and voltinism requirement for the developing embryos. Specifically, incubation aims at:

- ensuring that eggs of different varieties hatch on the designated dates and that moths of different varieties cross with each other at the right time to produce eggs of F_1 hybrid.
- enforcing uniform hatching to produce healthy newly hatched silkworms.
- Stabilizing the hibernating character of the bivoltine eggs, thus preventing eggs from hatching abnormally or without hibernation.

1.2 Methods of Incubation

On a large silkworm egg production farm, the proper size of the incubation room or chamber must be established according to the number and lots of the incubating eggs. The incubation room must have good temperature, humidity, heat protection, air circulation, easily adjustable light and should be easy to work in. Uniform temperature and humidity should be maintained in each room. Parent eggs for incubation are taken into the room by lots, adjusted to the proper temperature and humidity in accordance with the development stages of the embryo.

Incubation stands are usually laid in the middle of the room, with heating equipment at the four corners, and the heating sources kept at least one metre from the silkworm eggs. Light sources should be placed at a level either over or under the stands.

Incubation of bivoltine parent eggs in spring: The present bivoltine parent silkworms are bred for the purpose of producing hibernating eggs. Under the light, eggs are incubated at high or gradually increasing temperatures as they develop. When the embryo develops to a certain stage, light exposure should be prolonged and humidity increased. The requirements are shown in Table 1-1.

Table 1-1

Incubation criteria for bivoltine parent eggs in spring

Date in sequence	Embryonic development		Designated temp. (°C)	Designated humidity (%)	Difference between dry and wet bulbs of hygrometer (°C)	Light
	Marks	Stages				
Day released from cold storage	C_1 C_2	Before longest embryo stage Longest embryo stage	17	80	2	Natural light
1	C_2	Longest embryo stage	20	80	2	
2	D_1	Growing-in-thickness embryo stage	22	75	2	
3	D_1 E_1	Appendage development stage Early stage of appendage development	22	75	2.5	
4	E_2	Late stage of appendage development	24	75	2.5	18 hr./day, of under artificial light
5	E_3	Shortening stage	25-25.5	80	2	
6	F_1	Involution stage	25-25.5	80	2	
7	F_2	Final stage of involution	26	80	2	
8	F_3	Appearance of trachae stage	26	80	2	
9	F_4	"Eye-spot" stage	26	80	2	
10	F_5	"Blue egg" stage	26	80	2	Darkness
11 Hatching			26	80	2	

Notes:
1. For bivoltine races with multivoltine blood relationship, temperature should be adjusted to 0.5°C higher from the beginning of the E_3 stage.
2. Light exposure on the day of hatching begins at 04:00-05:00 hours.

Incubation of bivoltine parent eggs in autumn: The environmental requirements for the incubation of bivoltine parent eggs in autumn are about the same as those for bivoltine parent eggs in spring. However, the natural temperature in autumn is comparatively high and the air is dry. Incubation under such circumstances may cause the embryo to develop rapidly and consume much of the nutrients. The number of dead eggs during incubation will increase and the constitution of the newly hatched larvae will become weak. Therefore, it is advisable that incubation of the parent silkworm eggs in autumn should be carried out according to the criteria set out in Table 1-2.

Table 1-2

Incubation criteria for bivoltine parent eggs in Autumn

	Earlier stage	Later stage
Days	4	5-6
Embryo	Prior to E_3	E_3-F_5
Temp. (°C)	25	26-27
Humidity	70-80	80-85
Light	Natural	Light exposure increased 6 hours a day

Incubation of multivoltine parent eggs: Temperature and light do not have much effect on the voltinism of non-diapausing multivoltine eggs during incubation. Therefore, it is advisable to incubate the eggs at an average temperature of 26°C. During the "eye-spot" stage, the temperature for incubation should be regulated to at least 26.7°C but not higher than 29°C, and the relative humidity to 80-90 percent.

Measures for uniform hatching of silkworm eggs: Hatching of bivoltine parent silkworm eggs during incubation is often not uniform in spring. This causes problems in the rearing of the silkworms and affects the copulation of the male and female moths in the course of egg production. To ensure uniform hatching, the eggs should be kept at an intermediate temperature of 14-16°C before they are taken out from cold storage. Incubation begins when the embryo develops to C_1-C_2 stage. Egg selection should be conducted when the eggs reach the "eye-spot" stage. The eggs should be arranged in the order of eye-spot development. Incubation continues in the dark until the day of hatching, when light is turned on one or two hours before dawn to secure uniform hatching.

Keeping the eggs in darkness at the late stage of incubation prevents the early-maturing embryo from hatching and gives the late-maturing embryo time to continue to develop. After being kept in darkness for a certain period and suddenly exposed to light, both of these eggs may hatch simultaneously.

Cold inhibition of embryonic development for eggs during incubation or cold preservation of newly hatched larvae: If, for any reason, the brushing of the newly hatched larvae has to be postponed, the incubating silkworm eggs or the newly hatched silkworms should be preserved at a low temperature. Before the embryo develops to the E_3 stage (the shortening stage), the temperature should be kept at the objective temperature during incubation. However, when the embryo has reached the E_3 stage, the temperature should be raised according to the requirement of incubation. Cold storing is carried out when all the eggs have turned blue and the early hatched larvae can be found. The newly hatched larvae can also be cold stored to inhibit further development.

Eggs that have turned blue can be preserved at 5°C for 3-5 days. Those varieties likely to produce non-hibernating eggs can be preserved at 2.5°C for 1-3 days. Newly hatched larvae can be preserved at 10°C for 2-3 days.

The inhibition of the eggs from further development and the cold storing of the newly hatched larvae have some unfavourable effects on the physiology of the silkworms. Therefore, the shorter the time of inhibition, the safer it is for the health of silkworms. Furthermore, whenever the eggs or larvae are taken in or out of the preservation room, they must pass through an intermediate temperature for two hours.

1.3 Effect of Incubation Environment on Voltinism

Silkworms are broadly classified as univoltines, bivoltines, and multivoltines. Multivoltine sikworms can be sub-divided into diapausing and non-diapausing. Voltinism is the number of generations of insects produced in a year under natural conditions. Insects that produce only one generation in a year and then go into diapause are called univoltines. Those that produce two generations in a year before going into diapause are called bivoltines, and those that produce more than two generations in a year are called multivoltines.

Silkworms of different voltinisms present clear differences in duration of their development, in their constitution and in the quality of their silk. Generally, bivoltine has a shorter duration of development and stronger constitution than the univoltine, but the silk length is shorter and the quality lower. The multivoltine has the shortest duration of development, its silk is short and of poor quality, but its constitution is the strongest.

Univoltines lay only hibernating eggs, non-diapausing multivoltines lay only non-hibernating eggs. But bivoltines and diapausing multivoltines may produce either hibernating or non-hibernating eggs, according to the different environmental conditons

where they grow. The diapause of silkworm eggs is determined by the diapause hormone, secreted by the suboesophageal ganglia and transmitted through the body fluid to the ovaries of the mother moth, where it becomes functional and enables the embryo to go into diapause at a certain stage of its development.

Suboesophageal ganglia in any variety with different voltinism have the function of secreting diapausing hormones, but the secretion of the hormone is controlled by the brain. In normal conditions, the brain of the univoltine silkworm has the function of accelerating the secretion of the diapause hormone by the suboesophagael ganglia. The brain of the multivoltine silkworm has the function of inhibiting the secretion of the diapause hormone by the suboesophageal ganglia, whereas the brain of the bivoltine, under high temperature and light exposure during incubation, can accelerate the suboesophageal ganglia's secretion of the hormone, resulting in the production of hibernating eggs. Under low temperature and dark conditions during incubation, it can inhibit secretion and bring about production of hon-hibernating eggs.

The principal environmental factors affecting voltinism are temperature, light and humidity.

Temperature: When parent eggs of bivoltine silkworm races are incubated at a high temperature (25°C), their moths will lay hibernating eggs, and when incubated at a low temperature (15°C), they will lay non-hibernating eggs. An intermediate temperature (20°C) during incubation, will result in a mixture of hibernating and non-hibernating eggs. Since some of the present commercial bivoltine silkworm races have blood relationships with multivoltines, their voltinism is slightly different from that of the pure bivoltines, so environmental conditions have little influence on voltinism.

It should be emphasized here that the temperature during incubation has the greatest effect on voltinism. In other words, temperature in the incubation stage is the decisive factor determining whether bivoltine silkworms will lay hibernating or non-hibernating eggs thereafter, regardless of the environmental conditions during the larval and the pupal stages (Table 1-3).

Light: Light during egg incubation also influences voltinism. But the effect is not evident at temperatures above 25°C or below 15°C. Incubation under intermediate temperature with light produces a higher rate of hibernating eggs; without light, the rate of hibernating eggs is lower. It is obvious that the effect of light on voltinism is second to that of temperature.

Humidity: The effect of humidity on voltinism during incubation is next after that of light. It is insignificant at incubation temperatures above 25°C and below 15°C. At intermediate temperature, high humidity is favourable for laying hibernating eggs, while low humidity favours non-hibernating eggs.

Table 1-3

Voltinism of the progeny eggs as influenced by temperatures during the various stages of bivoltine parents

Incubation stage	Grown larval stage	Mounting pupal stage	Voltinism of eggs laid
High temperature (above 25°C)	High temperature (above 25°C)	High temperature (28°C)	Hibernating
	Low temperature (18°C)	Low temperature (20°C)	Hibernating
Low temperature (15°C)	High temperature (above 25°C)	High temperature (28°C)	Non-hibernating
	Low temperature (18°C)	Low temperature (20°C)	Non-hibernating
Intermediate temperature (about 20°C)	High temperature (above 25°C)	High temperature (28°C)	Mostly non-hibernating
	Low temperature (18°C)	Low temperature (20°C)	Mostly hibernating

Temperature, light and humidity affect the voltinism of silkworms at the stage between when the embryo begins to form the thoracic legs and when the head begins to be coloured. The basic requirement for the univoltines to lay hibernating eggs is a temperature above 20°C for incubation at this stage, and for bivoltines above 25°C. If incubated at a temperature below 15°C and kept in a dark and rather dry condition, most of the bivoltine races may produce most, if not all, non-hibernating eggs. Univoltine races may also lay non-hibernating eggs, but at the stage of embryonic development between A and C_2, temperature has basically no effect on voltinism. (Table 1-4)

Table 1-4

Influence of incubation temperature on eggs laid by bivoltine races in different embryo stages

Embryonic development stages at low temperature (15°C)	Embryonic development stages when moved from low temperature to 25°C	Days from transferring to high temperature till hatching (no.)	Voltinism of eggs laid
Involution completed	Body of newly hatched larvae formed	1	Total non-hibernating
Thoracic legs formed	Head begins to show colour	2	Total non-hibernating
A few just-formed blastodiscs	Involution is nearly finished	4	Total hibernation
Newly laid eggs	Involution stage	5	Total hibernation

CHAPTER 2

REARING OF PARENT SILKWORMS

2.1 Features of Parent Silkworm Rearing

Either for commercial silkworm egg production farms or for egg breeding stations, parent silkworms are silkworms whose eggs are to be taken for further egg production purposes. The cocoons thus harvested are called seed cocoons, from which the F_1 hybrid eggs are produced and distributed.

Most of the parent strains are pure lines, only a few being hybrids of different inbred lines. As the parent strains have a rather weak constitution, it is necessary to provide them with better feed, rearing environmental conditions and technical handling.

Parent silkworm rearing requires a good development, strong physique, and fine cocoon quality of the present generation to produce a large quantity and good quality commercial silkworm eggs of F_1 hybrid.

2.2 Principal Techniques for Parent Silkworm Rearing

Disinfecting the rearing room and appliances: Thorough cleaning and disinfection of the rearing rooms and appliances must be done as required before rearing of the parent silkworms. The room should be dried after disinfecting, and prepared for the brushing of the newly hatched larvae.

Brushing of the newly hatched larvae: For bivoltines, a suitable time for brushing the newly hatched larvae is at about 08:00-09:00 hours, or earlier for multivoltines. Methods of brushing are: leading the newly hatched larvae with thin tissue paper or striking them down onto the rearing beds. Both methods adhere to the principles of not injuring the larvae, and accurate larvae weighing and convenient handling. In brushing, one egg card is considered one batch and each batch contains 4-5 grams of newly hatched larvae. After brushing, the egg cards should be retained for the inspection of the pebrine pathogen of the egg sheels.

Feed: The quality of mulberry leaves affects the economic properties of the present generation of silkworms, such as silkworm physique, cocoon quality, egg quality, voltinism and moultinism. Furthermore, the leaf quality also affects the rearing result of the following generation.

The infant stage (first to third instars) is the stage of body development in which the larvae grow fast. If the feed is not good, the larvae will suffer from insufficient supply of nutrients and their growth will be greatly affected. In which case it is difficult to obtain a successful crop even if a good supply of leaves is given at the later stage.

The grown stage (fourth and fifth instars) is the stage at which nutritional matter is accumulated greatly, thus ensuring the growth and development of the reproductive cells and the silk glands. It has been proved by experiments that the number of eggs formed has a close correlation with the nutrients absorbed by the larvae in the fourth and fifth instars. If the larvae are malnourished, they will lay less and smaller eggs, which will most likely be susceptible to adverse climatic conditions.

The quality of mulberry leaves is closely related to the varieties of the mulberry trees, topography of the mulberry garden, soil, density of planting, application of fertilizers and the form and time of pruning and cutting. Silkworm egg farms should have a mulberry garden used exclusively for the infant larvae, and should choose good varieties of mulberry trees suitable for the rearing of parent silkworms. The mulberry garden of the egg farms should have sandy loam soils in a high location with sufficient sunlight, good ventilation and proper management. The land should have good drainage and irrigation.

In order to maintain the leaf quality for the rearing of parent silkworms, care should be taken in the harvesting transport, storage and arrangement of the leaves. The leaves must be kept as fresh as possible.

Regulation of climatic conditions: Since climatic conditions, including temperature, humidity, air circulation, gases and light, affect the growth and development of silkworms, these must be properly regulated.

Temperature: The rearing temperature directly influences the physiology of silkworms, in nutrient absorption, digestion, blood circulation, and respiration. Generally, the temperature range for normal growth is 18-30°C, but the optimum temperature range is 22-28°C. In the tropical and sub-tropical zones, the optimum temperatures for the present bivoltine parent strains are as follows: for the infant larvae, 27-28°C; for the grown larvae, 24.5-25.5°C. In the case of multivoltine parent strains, the rearing temperature is a little higher than that for the bivoltines. For grown larvae, the temperature should not be lower than 26.5°C.

Since the natural temperature in the tropical and sub-tropical zones in the rearing season is often above 28.5°C, it is necessary to lower it.

Humidity: Humidity indirectly influences silkworm physiology through the freshness of the feed and the sanitation of the rearing beds, and thus the growth and health of the silkworms. The optimum relative humidity for rearing parent silkworms is as follows: the first and second instars 80 percent; for the third instar 75 percent; for the fourth instar 70-75 percent; for the fifth instar 65-70 percent.

Quality and circulation of air: In the rearing room, some gases, such as carbon dioxide and ammonia, are released by the fermentation of the refuse leaves and faeces in the rearing beds and by the respiration of the silkworms. Harmful gases, such as carbon monoxide

and sulphur dioxide emitted by the burning of fuels, can harm the physiological functions of the silkworms when they reach a certain level.

The respiration rate of the infant larvae is low. They can stand carbon dioxide, but they cannot resist the poisonous gases, such as carbon monoxide and sulphur dioxide. However, the opposite is true at the grown stage. At the mounting stage, if the mature larvae come into contact with too much carbon dioxide, the number of non-cocooning larvae will increase.

Infant larvae have no particular requirement for the circulation of air. Periodic ventilation is quite all right. At the grown stage however, the rate of respiration is high and a large amount of mulberry leaves is consumed, therefore the humidity in the room is high and the air is easily polluted. Great care should, therefore, be taken to allow free circulation of as much fresh air as possible to accelerate the metabolism of the worms and to promote their health. However, at the time of ventilaton, it is important to prevent the mulberry leaves from withering.

Light: Light has no evident effect on the health of the silkworms, the quality of cocoons and the number of eggs laid. It is quite all right to keep parent silkworms under natural conditions, i.e., brightness during the day and darkness at night. However, in the case of those races that easily produce non-hibernating eggs, at the grown stage darkness can effectively reduce the production of non-hibernating eggs.

Examination of the undergrown larvae: To ensure that the commercial silkworm eggs produced are free from pebrine disease, undergrown larvae, sick larvae, dead larvae, and those which have fallen to the ground from mountages from different batches and in different instars, should be subjected to microscopic examination. If perbrine spores are present in the undergrown larvae before the third moulting, the whole batch should be rejected. If the larvae after the fourth moulting are found to be infected with pebrine spores, whether to reject or not is determined in accordance with the number of spores and the degree of infection. If infection is serious, the whole batch should be rejected. If it is slight, the batch should be separated and used for reeling cocoon rearing. The room and appliances used to rear the infected batches should be thoroughly disinfected.

Regulating emergence of the moths for mating: In rearing parent silkworms, it is always important to observe and compare the development of the parental larvae for mating. If there is any deviation in the actual duration of development from what is expected, regulations should be done by increasing or decreasing the rearing temperatures, or by moving up or delaying the time of feeding. Generally speaking, for the slow-developing varieties, the rearing temperature can be raised 1°C higher than the designated one, and for the faster ones it can be lowered 1°C. At the time of mounting, it is better to keep the faster-developing larvae in a cooler mounting room and the slower ones in a warmer one. However, in the course of regulation, attention must be paid to the influence upon the health and voltinism of the worms.

2.3 Mounting and Harvesting

The mounting of the parent silkworms should adhere to the principle of proper maturity and should not be crowded. Silkworm maturity is recognizable by the translucent appearance of the anterior part of the larvae; it raises its head high, moving about and seeking a position for cocooning. Parent silkworms are usually mounted at a stage when they become a little over mature, never when they are immature. Immature silkworms, because of not being well fed, not only will delay the spinning of silk for cocooning, but also will become weak and lay fewer eggs. However, they should not be too over mature. Over maturity may lead to the production of double and deformed cocoons.

In practice, when mounted at night or when there are only a few worms maturing, the worms can be a little over mature; but if mounted at the peak of the mature stage or at noon, it is preferable that the worms be a little immature.

Collecting mature silkworms and mounting them onto the mountages is a laborious job. Labour distribution should be well organized. The larvae should be mounted when they are collected and should not be piled up. Care must be taken to clearly identify the varieties and the time of mounting. Confusion should be avoided.

Mounting parent silkworms should be less dense than that of silkworms reared for reeling cocoons. The rearing room should be well ventilated and adequately heated to drive away the excess moist air.

The protective temperature in the mounting stage for the bivoltine centres around 24.5°C and that for multivoltines around 26.5°C. The difference between the dry and wet bulb of a hygrometer is 3°C. The mounting room should not be too bright; it should be kept in a slightly dark and quiet condition.

Time for cocoon harvesting varies with the silkworm varieties and their protective temperatures in the mounting stage. Generally speaking, cocoon harvesting for the bivoltines begins 6 days after they have been mounted and for the multivoltines, three to four days. While harvesting, the cocoons should be laid aside separately according to their varieties and mounting dates; double cocoons, fly-maggot-parasitized cocoons and defective cocoons, sorted out. The silkworm pupae should be handled gently to avoid any injuries.

Cocoon harvesting at the early stage prevails in eastern China. That is to say, cocoons are harvested when the silkworms have finished their spinning of silk and are about to pupate. With a protective temperature of 25°C and a mounting period of 60-70 hours, cocoon harvesting at the early stage achieves effective results in enhancing egg production of the bivoltines, whose pupal bodies are relatively larger.

2.4 Effect of Rearing Environment on Voltinism

During the rearing period, temperature, light and nutrients also have certain influence on voltinism, but less than in the incubation stage (see Table 1-3).

The investigation result shown in Table 1-3 is the typical status of the bivoltines. As some of the present bivoltine races have blood relationships with multivoltines, their voltinism is unstable. In order to stabilize the hibernation of the bivoltines, it is still necessary to pay attention to the control of temperatures in the rearing stages, though high temperature and exposure to light are necessary measures during incubation. Only by maintaining high temperatures (above 26°C) for the infant larvae and low temperatures (below 24°C) for the grown larvae, can hibernating eggs be obtained.

Light also has some effect on the voltinism of the silkworms during the rearing stage. When it is bright in the infant stage, the worms produce mostly hibernating eggs, and in the grown stage non-hibernating eggs. If reared under dark conditions, they tend to lay more non-hibernating eggs..

Moreover, the quality of the feed can also affect the voltinism. Feeding with nutritious leaves helps to produce hibernating eggs, while poor feeds or feeding with tender leaves tends to produce non-hibernating eggs.

The reason why the effect of environmental conditions is so evident on the voltinism of the bivoltine races can be explained by their life cycle under natural conditions. (Figure 2-1).

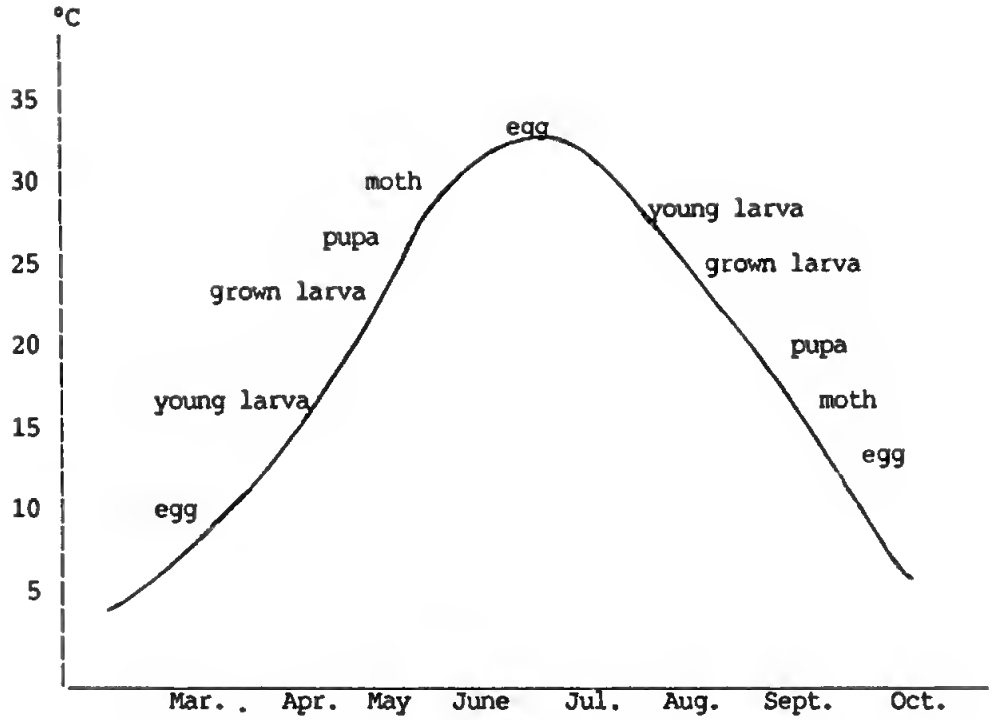


Figure 2-1

Natural life cycle of bivoltine silkworm races in the temperate climate

CHAPTER 3

INVESTIGATION AND PRESERVATION OF SEED COCOONS

Seed cocoons are the source material used to produce silkworm eggs. The quality of seed cocoons is related to the quality of silkworm eggs and the success of the rearing of the F_1 hybrid silkworm. Therefore the seed cocoons harvested have to be subjected to strict examination. Only those that conform to the standard can be used for egg raising. The cocoons that quality should be preserved reasonably to promote natural morphological development in order to ensure high yield and high quality eggs.

3.1 Quality Investigation of Seed Cocoons

Criteria for the quality of seed cocoons: As the climatic and geographical conditions vary greatly in different parts of the world, the varieties of silkworms are different, and so is the quality of cocoons. Even the same variety of silkworm reared in different areas may vary in characteristics. Therefore the standard for inspecting the quality of seed cocoons varies with different places.

Methods of inspecting the quality of seed cocoons

Items for and time of inspection: Inspection for the quality of seed cocoons includes the following four items: the amount of cocoons harvested, the cocoon shell weight, the cocoon shell ratio, and the dead cocoon ratio. The rearing batch is the unit for all four.

The time for inspection is as follows: For Spring and late Autumn crops, it is 7-10 days after full mounting; for early Autumn and mid-Autumn crops, 6-8 days after full mounting. For varieties with a shorter duration of the pupal stage, such as Dong 34, inspection can be a day earlier. If the interval between mountings is too long, inspection can be done section by section, the results then based on the average.

Method of inspection

Inspection for the amount of cocoons harvested: The reproductive cocoons, double cocoons and spoiled cocoons should be weighed separately and the total weight taken. The average cocoon yield of 10 g ants should be counted and then 1 kg of each kind of cocoon taken and their numbers counted.

Method of sampling: 5-6 kg of cocoons should be taken at random for sampling. The floss of the cocoon should be removed. They should be thoroughly mixed and then laid flat. The cocoons should be divided into four parts by two diagonal lines. Any two parts on the diagonal angles should be taken and 2 kg weighed for various examinations. The two other parts are to be taken as the sample for the investigation of selected cocoon rate.

Analytical check for dead cocoons: Inspection of dead cocoons in the reproductive cocoons - if the ant weight of the rearing lot is over 80 g, 1 000 cocoons should be checked, and if below 80 g, 500 cocoons should be checked.

Inspection of dead cocoons among the double cocoons and spoiled cocoons: If the ant weight of the rearing lot is over 80 g, 500 double cocoons should be checked, 0.6 kg spoiled cocoons; if below 80 g, 250 double cocoons, 0.5 kg spoiled cocoons.

The cocoons should be cut open for inspection of dead cocoons of different kinds. The death rate of the entire lot should be totalled. If c is taken as the weight of the different kinds of cocoons, n as the number/kg of different kinds of cocoons, d as the death rate of different cocoons, and D as the dead cocoon rate of the entire lot, the dead cocoon rate of the entire rearing lot can be calculated by the following formula:

$$D = \frac{> cnd}{> c\ n} \times 100$$

Dead cocoons include any of the dead worms, dead pupae, infected worms and pupae, part-moulted pupae (covering the thorax or more than two segments on the anterior part), unmoulting pupae, or pupae with three segments on the abdomen turning black, and the hairy-leg worms (unpupated larva) after 6 days on the mounting in the cocoons.

Bloody pupae, parasitized pupae by fly maggots, and muscardine infected pupae need not be examined. But in the parent silkworm rearing batch, the record of these three items should be taken as a grading index for seed cocoon purchase.

Inspection of the quality of cocoons: 120 cocoons should be taken from the samples at random. They should be individually opened, their sexes identified, and 50 male and 50 female cocoons selected. The whole cocoon and the cocoon shell should be weighed and the cocoon shell rate calculated.

$$\text{Cocoon shell rate \%} = \frac{\text{Cocoon shell Weight} + \text{Cocoon shell Weight}}{\text{Whole cocoon Weight} + \text{Whole cocoon Weight}} \times 100$$

Investigation of the casting cocoon rate: Remove 2-2.5 kg cocoons from the other half of the samples, and cast the defective cocoons. Count the casting cocoon rate as a criterion of the casting cocoon rate of that whole lot. The rate should not be lower than 3 percent for the spring crop and not lower than 4 percent for the autumn crop.

If the result of any of these examinations is not up to standard, two more examinations may be taken from other samples. If the average result of the three examinations is still not up to standard, the whole lot of cocoons should be rejected.

3.2 Selection of Seed Cocoons

After quality inspection, rearing lots of seed cocoons should undergo individual selection, and those which do not conform to the racial characteristics of the parental races should be discarded. This maintains the uniform economical characteristics of the variety and raises the quality of the cocoons.

Defective cocoons are:

- **Thin cocoons** - Their shells are extremely thin and can be easily crushed.
- **Malformed cocoons** - Irregularly shaped, do not conform to the racial characteristics of the original variety.
- **Fluffy cocoons** - Loose cocoon shell, but not shriveled; soft and loose like cotton when touched.
- **Coloured cocoons** - Light yellow, yellowish green, or other colours.
- **Other defective cocoons** - Sharp-point cocoons, broken-end cocoons, undersized cocoons, bee-waist shape cocoons, etc.

Cocoons are defective due to genetic or environmental factors. Those affected by genetic factors, such as coloured cocoons, fluffy cocoons, bee-waist cocoons and sharp-point cocoons, should be discarded. Malformed cocoons, thin cocoons, broken-end cocoons, etc., are generely caused by environmental factors. It is preferable to discard them so as to retain the uniform characteristics of the races.

3.3 Preservation of Seed Cocoons

Temperature: It is of the utmost importance that seed cocoons be preserved at proper temperatures. In the case of bivoltines, the optimum temperature for preservation is 24°C, at which the eclosion rate is the highest, the number of eggs laid by a mother moth reaches the maximum, and the rates of unfertilized eggs and dead eggs are low. In the case of multivoltines, the cocoons should be kept at 26°C. If the seed cocoons are kept at 30°C, not only will the number of pupae with flacherie disease increase and the rate of moth emergence decrease, but the number of unfertilized eggs, dead eggs and non-hibernating eggs will increase significantly. The effect of high temperature is most evident in the stages between mounting and pupation. Male pupae suffer most. If the temperature is lower than 20°C, moth emergence rate will decrease, the number of eggs laid will fall sharply, unfertilized eggs will increase and the female pupae will suffer the most.

The effects of different temperatures in the pupal stage on eggs are shown in Table 3-1.

Humidity: The optimum humidity for the preservation of seed cocoons is 75-80 percent. Generally, at the range of 65-90 percent, humidity has no evident effect on the health of the moths, on the number of eggs laid, the number of unfertilized eggs, or the next generation of silkworms. In practice, when eggs are produced in autumn, the air is often too dry. When the humidity is lower than 60 percent, the pupae may develop slowly, the percentage of eclosion (emergence) will decrease, and the number of moths failing to mate will increase. They are easy to separate, even when they have had the opportunity to copulate. If it is too dry at the later stages of seed cocoon preservation, the effect will be more serious. Therefore, around the time when early emergence of the moths appears, humidity should be adjusted according to actual conditions.

Table 3-1

Relations between different preservation temperatures in pupal stage and egg laying

Preservation temperature (°C)		Number of moths investigated	Number of eggs laid		Number of good eggs		Number of unfertilized eggs		
Before pupation	After pupation		Per moth	index	Per moth	index	Per moth	Percentage	Index
25	25	41	576	100	552	100	24	4.20	100
20	20	24	480	83.33	448	81.17	32	76.68	159.05
30	30	19	246	43.63	172	31.09	74	30.13	717.38
20	30	13	394	68.44	311	56.40	83	21.05	501.19
30	20	23	326	57.60	300	54.34	26	8.03	191.19

Light: In the period of preserving the seed cocoons, care should be taken to maintain brightness in the daytime and darkness at night. It is important that the night be completely dark and that the cocoons be exposed to light before dawn on the day of eclosion, thus making the moths emerge earlier and in conformity.

Air: As the pupae develop, their rate of respiration gradually increases. Large amounts of carbon dioxide are released when the compound eyes are coloured. At this time, ventilation is necessary. Air should be kept fresh in the rearing rooms.

3.4 Observation of the Development of the Pupae and Regulation of Mating

To regulate the mating of the moths, it is necessary to observe the development of the pupae. Temperatures should be regulated in accordance with the degree of pigmentation of the compound eyes, antennae, and their bodies. Thus, the moths of the two varieties for mating will emerge simultaneously.

Correlation between pigmentation and development of the pupae: Generally, pigmentation and development of the pupae are correlated as follows:

- The start of pigmentation of the compound eyes marks the halfway point between mounting and emergence.
- The compound eyes become black at the two-thirds point.
- When the antennae turn dark black, moths will emerge in two or three days.
- When the pupa's body becomes loose and soft, loses its lustre, and becomes shrivelled, moths will emerge the following day in the case of the Chinese race and two days later for the Japanese race (Figure 3-2).

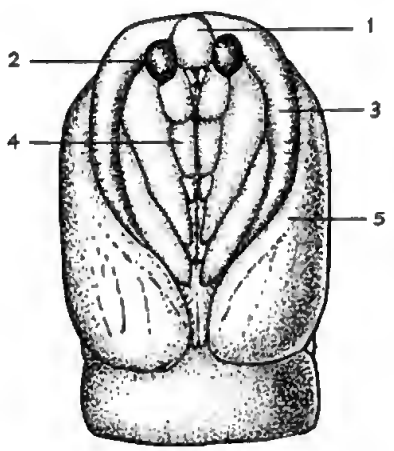


Figure 3-1
Head and thorax of the pupa (compound eyes and antennae)

1. Firons
2. Compound eyes
3. Clypeus
4. Labrum
5. Thoracic legs

Regulation of the emergence of the moths: Generally speaking, the temperatures for the preservation of the pupae from mounting to the emergence of the moths, are in the range of 21-27°C. Whenever the temperature rises or falls 1°C, the emergence of the moths may surge ahead or delay one day. If the temperature rises or drops 2°C, it may surge ahead or delay two days. A lower temperature should often be used for pupae developing faster and vice versa. Care must be taken to observe regularly the development of the pupae and to regulate the temperature in order that the moths of the two varieties for mating will emerge on the same day and in uniform quantities.

Cold storing of the seed cocoons (pupae): There are two periods for cold inhibition of silkworm pupae: (a) two to three days after pupation when the compound eyes begin to pigment, and (b) a day before the emergence of the moths. Cold storing should be done in the evening. The time limitation of cold storing for males is 4-5 days, and for females 2-3 days. The temperatures range from 2.5 to 7.5°C, with 5°C as the median.

Cold storing of the seed cocoons may lower the percentage of moth emergence, the number of non-laying moths may increase, and the number of eggs laid may decrease; the percentage of unfertilized eggs may also increase. Therefore, seed cocoons should not be stored in cold unless it is absolutely necessary.

CHAPTER 4

SEX DISCRIMINATION (SEXING)

Sex discrimintion, or sexing, is an important technical measure in the production of F_1 hybrid silkworm eggs. It is the separation of the female from the males within the same variety. There are three methods: (1) sexing by the external characteristics of the larvae, (2) sexing by the external characteristics of the pupae, and (3) sexing by the external characteristics of the cocoons.

4.1 Sex Discrimination of the Larvae

On the ventral side of the abdomen of the female larva, on the eighth and the ninth segments, there is a pair of small round spots on both the left and right sides. These are referred to as Ishiwata's foreglands and Ishiwata's hind glands. In the male larvae, a small milky white follicular body called Herold's gland (Figure 4-1) is present in the centre of the ventral side on the junction of the eighth and the ninth segments. By the observation of the different characteristics of the reproductive glands of the male and female larvae, sex discrimination of the larvae can be done with the naked eye from the late fourth instar to the fifth instar.

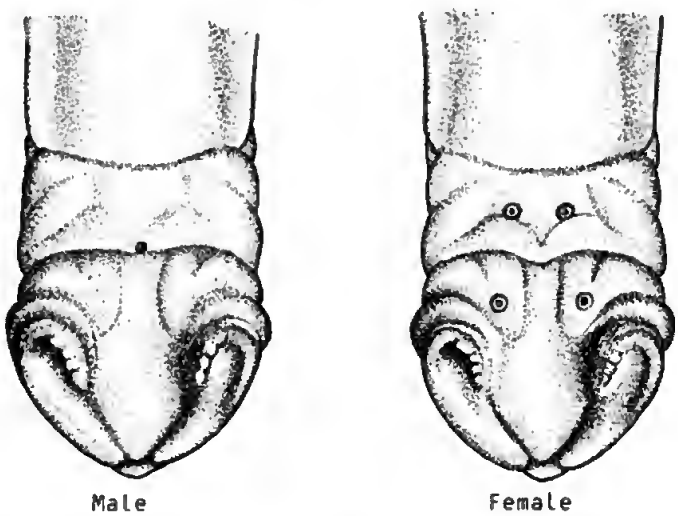


Figure 4-1
Characteristics of the reproductive glands of male and female larvae

4.2 Sex Discrimination of the Pupae

The female silkworm pupa is larger than the male pupa; the colour of its pupal skin (cuticle) is lighter, and the posterior abdominal segment is more rounded in shape. Ventrally, in the centre of the eighth abdominal segment, there is an X-shaped mark extending from the anterior to the posterior margins of the segment.

The male silkworm pupa is smaller than the female; the last abdominal segment is more or less pointed, and in the centre of the ninth ventral segment, there is a small brown spot, the Herold's gland.

According to the external features — the body shapes and the prominent markings of the reproductive glands — it is possible to distinguish the sexes of the silkworm pupae (Figure 4-2).

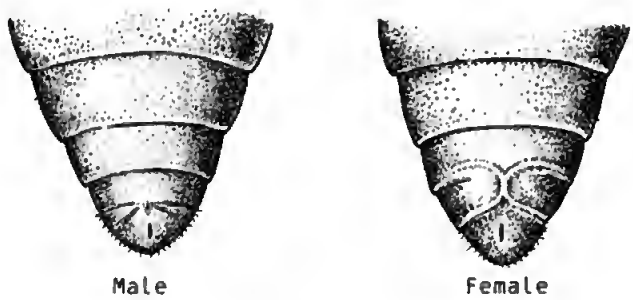


Figure 4-2
External shapes and characteristics of the reproductive glands of male and female pupae

4.3 Sex Discrimination of the Silkworm Seed Cocoons

Since female cocoons are usually larger and heavier than males of the same race, especially among the multivoltine races, use can be made of these differences to separate the female cocoons from the male cocoons by weighing. Sexing machines are popularly used for this purpose in the silkworm egg production farms in Guangdong Province, China. The sexing machine can make the work more efficient, and the moths can emerge from their own uncut cocoons. When moths emerge from their uncut cocoons, the average rate of egg production can be approximately 20 percent higher than when the cocoons are cut open, because the risk of injury to the pupae is reduced.

Before employing mechanized sexing, a few samples of cocoons are taken at random from the same batch of seed cocoons that were mounted on the same day. The weight of a criterion cocoon is determined based on the ranges of weight between the female and male cocoons. This

will be used as a differentiation weight for sex separation of seed cocoons: cocoons that are heavier than the criterion belong to the female group, those lighter to the male. Cocoons of the same weight as the criterion cocoon belong to a mixed group of both sexes.

The correct evaluation of a criterion cocoon has considerable effect on the work as a whole. A criterion weight must maintain a rate of sexing of 90 percent or higher. At the same time, the sexing rate of the mixed group should be controlled to 10 percent or less. If the requirements stated above cannot be met, the application of mechanized sex separation will be of no significance.

The cocoon sexing machine (Figure 4-3) consists of a rotary main axis, the upper part of which is fitted with a perforated disc. Attached to this are 16 small lever-type steel arms, hung at regular intervals.

When the machine is in operation, a small electric motor sets the hollow disc rotating and the small steelyards rotate along with it. According to the lever principle, the rotating steelyards can measure the weight of the cocoons automatically. These have three positions for the different weights of the male and female cocoons (Figure 4-4): one, the beam of the steel arms is level; two, it inclines toward the outer part of the disc; three, toward the inner part of the disc.

Figure 4-3
Cocoon sexing machine

The small steel arms in three positions control the positions of the pans of the steel arms in different heights. Cocoons loaded in

the pans will be scraped off in different positions and thus divided into three groups, the male, female, and mixed male and female.

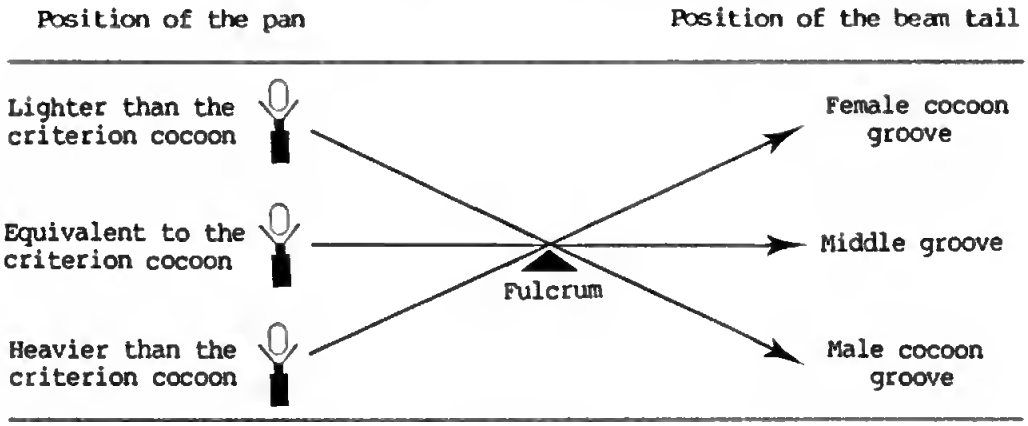


Figure 4-4
States of the steel arms weighing cocoons automatically

Efficiency of the cocoon sex separating machine: This cocoon sex separating machine rotates at a speed of 14.5 cycles per minute. If a certain silkworm race has an average of 740 cocoons per kilogram, the efficiency of the cocoon sex separating machine can be calculated as follows:

No. of cycles of the rotation of the round disc per hour:
60 x 14.5 = 870 cycles
No. of cocoons passed through the machine per hour:
870 x 16 = 13 920 cocoons
No. of seed cocoons separated per work day:
13 920 x 8 = 111 360 cocoons
No. of seed cocoons converted into weight:
111 360 : 740 = 150.5 kg.

At present, in practical production, the work efficiency of the sexing machine differs rather greatly from the theoretical efficiency, because the machine must be adjusted prior to every cocoon sex separating operation. Furthermore, a worker dropping cocoons onto the pans manually is much slower than the rotating speed of the machine. If the machine can be improved to a kind of combined device (including deflossing and automatic serving of cocoons), the efficiency of mechanized cocoon sex separation would be greatly enhanced.

Sexing by weighing individual cocoons: A single-cocoon scale (Figure 4-5) can also be employed for sex separation. This is also designed according to the lever principle. Although the rate of efficiency is much lower, it is easy to work, and it is very accurate. It is still of some practical value in production.

Figure 4-5
Single-cocoon scale

CHAPTER 5

EGG LAYING

5.1 Methods of Egg Laying (oviposition)

There are two basic methods of egg laying: segregated egg laying and mixed egg laying. Segregated egg laying can be sub-divided into Pasteur's method, which prevails in China and Japan and the cellular bag method, which is usually practised in European countries. Mixed egg laying can be sub-divided into flat card laying and loose formed egg laying. The method chosen has a bearing upon the grade of the silkworm egg production, the characteristics of the race, the conditions of the attacks of silkworm diseases, etc. For example, the production of grandparent eggs of F_1 hybrid and of parent race every imago (moth) must take the pebrine test, so segregated egg laying is preferred. But for commercial eggs or eggs for silkworm production, in which not all of the moths are required to undergo the pebrine test, mixed egg laying may be adopted. Take, for instance, some silkworm races in Europe. Owing to the degradation of the Filippi's or Lyonnet's glands, the eggs laid lack viscosity. This makes the cellular bag egg laying method imperative.

Flat card method: This type of mixed egg laying in which a certain number of female moths are permitted to lay eggs in a definite area on a sheet of kraft. It is simple, convenient, and labour saving, except that it does not permit individual selection or elimination of the silkworm eggs. The modern flat card method is one of the commercial egg laying practices that require that the eggs used be, on the whole, free of pebrine disease. The specification is that each egg card have an egg laying area of 21 cm x 16 cm = 336 cm^2. Moreover, there is a difference between a single and a double flat egg card (Figure 5-1). The standard quantity of eggs on each egg card is the quantity required for the eggs to fully occupy the egg card in one grain layer. The number of mother moths placed in each egg card varies with the race, area, season and quality of the seed cocoons. For example, in Guangdong Province, each egg card accommodates 60-70 mother moths of the common bivoltine race, and 75-85 mother moths of the multivoltine race.

Loose formed method: For commercial silkworm eggs, the loose formed method is a more advanced method. In Europe, this method is obligatory because of varietal characteristics. In Japan, all silkworm eggs for cocoon rearing are produced employing this method, and it is commonly used in the eastern provinces of China also. The loose eggs are contained in boxes, each containing 12 grams (Figure 5-2).

Loose eggs are of superior quality to eggs on cards because this method facilitates individual egg selection and elimination of defective eggs. Moreover, thorough surface disinfection is possible and the quality of the silkworm race is thus improved. At the same time, the quantity of eggs produced per unit is precise and uniform, which is advantageous for projected production. The loose formed method is better for preservation and transport. However, the drawbacks of this method are that the eggs are shaken too violently during transport and in the process of incubation the eggs must be stirred, and the harvesting of the newly hatched larvae is not very handy.

Variety	Voltinism	Date of Oviposition	Date of acid treatment	Date of putting in cold storage	Date of leaving cold storage	Name of the Silkworm egg production Farm

Figure 5-1
Flat card egg laying (a double flat egg card)

Figure 5-2
Loose formed egg laying

Pasteur's method: This method is adopted for the production of original (parent) strains and of grandparent silkworms of F_1 hybrid. Owing to the convenience of carrying out microscopic inspection of individual moths and the elimination of diseased batches, this method is employed to render thoroughly disease free for parent strain and grandparent silkworm of F_1 hybrid. The area of each egg card for the parent strain is 32 x 18 cm^2, divided into 28 partitions, in each of which is placed or seated a single mother moth (Figure 5-3). After oviposition, the moths are put into a box labeled with their serial numbers to await microscopic inspection. For the laying of the grandparent silkworm eggs, each egg card consists of 14 moth seats. In addition to making strict microscopic inspection of the moths, it is necessary to investigate the life span of the female moths to serve as reference in the selection and elimination procedures.

Variety	Voltinism	Date of Oviposition	Date of acid treatment	Date of putting in cold storage	Date of leaving cold storage	Name of the Silkworm egg production farm

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28

No. _____

Figure 5-3
Chart showing Pasteur's method of egg laying

Cellular bag method: The cellular bag method also belongs to a type of segregated egg laying. Small cellular bags are made of thin cotton cloth or porous kraft into which the female moths are placed to lay their eggs. This cellular bag type of egg laying is particularly favourable to certain silkworm races — the mother moths of which lay non-gluey eggs. This is the system adopted in the USSR. As to the number of moths in a cellular bag, generally for the parent strain, one moth in a bag, but for commercial silkworm eggs, two moths occupy one bag.

5.2 Preparations for Egg Laying

Egg laying is an intense and tedious job that requires precision timing for its several stages. Everything should be well prepared in advance to avoid confusion, errors or other hindrances.

The houses for egg laying are generally composed of an emergence room (the seed cocoon preservation room), mating room, egg laying room, low-temperature room and silkworm egg preservation room. In commercial egg production farms where parent silkworms for hybridization are reared themselves, these rooms can also be used as rearing rooms or mulberry storage rooms. But the rooms for the rearing and egg laying of parent silkworms should be reserved exclusively for these purposes.

The rooms for emergence, mating, egg laying and preservation of silkworm eggs should be equipped with air conditioning devices to regulate the temperature, humidity and ventilation. The rooms should be uniformly bright during the day time and dark at night; intense light and strong wind should be avoided. For the mating and egg laying rooms, a quiet atmosphere is preferred. The egg laying room should have conveniently controllable light and should be equipped with ventilation facilities.

In principle, the temperature of the cool room is to be maintained at 10°C. The thermal insulation must be checked and tested ahead of time.

The perforated cotton cloth for covering the cocoons, the bamboo trays for mating and the wooden boards and frames for egg laying should be cleaned and sterilized before use. All the tools needed should be prepared in correspondence with the highest rate of daily emergence, or about 50-60 percent of the rate of the whole batch of eggs laid. Other equipment includes the draft sheets, female moth boxes, male moth cases, and the wired stand for holding the silkworm egg cards.

5.3 Egg Laying Techniques

Eclosion or emergence: During seed cocoon preservation, the pupa develops and metamorphoses gradually until it finally emerges as a silkworm moth. When it is approaching emergence, the moth inside the cocoon spits out a kind of basic fluid that dissolves the sericin and dampens and softens the silk filaments of the cocoon shell. Then, pushing through the cocoon shell with its head, the moth crawls out of the cocoon. The scaly hair on the body surface of the freshly emerged moth is moist, the body flexible, wings small and curled. The moth stays motionless. Soon afterwards, the scaly hair gradually dries, the body fluid enters the veins of the wings, and the wings begin to spread apart. Not until now do activities commence, especially with the male moths. They will continuously flap their wings and crawl around in an exceedingly active state while looking for female moths to mate.

Shortly before it emerges, the pupa wriggles once in a while with an audible sound. When metamorphosis is complete, the moth body and the pupal skin gradually separate from each other. The pupa loses

its original lustre and becomes dark brown with no elastic wrinkles on the body surface. Before emergence, the pupal body is delicate and soft to the touch. This is the chief characteristic of this developmental stage.

The univoltine race has a longer pupal period than the bivoltine, and the multivoltine has the shortest pupal period. As to the conditions of the preservation environment, within the range of optimum temperatures, the higher the temperature the faster and more uniform will be eclosion. The lower the temperature, the slower and irregular will be eclosion. But too high a temperature will result in the prolongation of the pupal period. Under optimum environmental conditions, the pupal duration of the Chinese univoltine race is 14 days; the Japanese univoltine is 17 days. The Chinese bivoltine race takes about 12 days; the Japanese bivoltine race approximately 14 days. The Chinese multivoltine races of Guangdong Province, however, take only about 10 days.

Under normal preservation conditions, exposure to light begins at dawn, at about 04:00 hours, and emergence essentially ceases at 08:00 hours on the same day for the bivoltine and at 06:30 hours for the multivoltine races.

Emergence takes longer in the univoltine races: in the bivoltine races it is next in duration, and in multivoltine races it is the shortest. The number of moths to emerge daily is not uniform either, but it has a definite pattern. A good knowledge of the pattern of emergence is of particular importance to the projected egg production work. A technician for egg production must be aware of this pattern.

Table 5-1

Rate of daily emergence

	(Percent)						
Race	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Univoltine	3.1	12.4	23.5	28.5	22.0	8.0	2.5
Bivoltine	3.2	22.0	51.6	20.4	2.8		
Multivoltine	29.5	47.8	20.7	2.0			

For the convenience of picking moths and preventing the moth urine from contaminating their bodies, a piece of cotton cloth should be used to cover the cocoons before emergence. In order to make appropriate arrangements for daily egg laying, it is advisable to induce simultaneous emergence. For this reason, light exposure should begin at 04:00 hours, and rearers should be specially assigned to pick out males quickly. That is, they should pick out immediately the male moths commingled with the female moths because of error in sex discrimination. In case copulation is detected, the copulating female moth should be discarded.

Moth collecting: After the light is turned on and photosensitivity has commenced for two to three hours and the scale hair of the emerged moths has become dry and the wings are fully stretched, moth collecting may begin. The male moths, being very active, flap their wings and crawl around, and these should be collected first and attached with a label indicating the name of the race, the number of the batch, and the date. They should be temporarily stored in a cool, dark place to inhibit their activities and reduce their energy consumption. The female moths are then collected in turn and spread evenly on a tray, leaving ample space in between for the distribution of male moths for mating.

When the male moths smell the scent of the alluring glands of the female moths, they will become highly stimulated and very animated. This not only causes the male moths to waste much of their energy, but also makes a great many of them drop to the floor from the trays, which creates difficulties in the work of picking. Therefore, before moth emergence, the two sexes of silkworm cocoons should be stored in separate rooms. Moreover, cases for storing the male moths and trays for holding the female moths should be fixed.

Under normal conditions, the work of moth collecting is generally carried out from 06:00 to 07:00 hours.

Moth selection: Moth selection is important for improving the quality of the silkworm eggs. At every stage in the process of egg raising, defective moths should be eliminated. Before the moths mate, it is necessary to carefully sort and eliminate the defective female moths. The morphological characteristics of defective moths are as follows (Figure 5-4):

- Body segments too long, body compact, body either bulky or midget in comparison with normal moths.
- Abnormal body shape, darkened or speckled with black on ventral segments and wings, etc.
- Abnormal or underdeveloped wings.
- Scaly hairs dropped, inert.
- Inactive, incapable of copulation.

Within a batch of cocoons, a small quantity of exceptionally early or late emerging moths should be eliminated and not included for egg deposition.

Figure 5-4
Defective moths

Copulation: As the work of moth collecting terminates, preparation for mating should begin at around 08:00 hours. First, the female moths are evenly distributed on trays and then male moths are cast on them and allowed to mate naturally. There should be about 20 percent more males than females. After about 10 to 20 minutes, pairing will begin naturally. The unpaired moths are picked out and gathered in a separate tray. The paired moths in the process of copulation should be arranged evenly with adequate spacings in between to avoid interruption and decouplings.

Ample time must be allowed for copulation. If it is too short, egg laying will be late and slow, fewer eggs will be laid, and more of them will be unfertilized. On the other hand, if copulation time is too long, decoupling will result in immediate ovulation and great loss. As to the duration of copulation, from the standpoint of fertilization, two hours is enough. In practical production, it usually lasts four to five hours. Besides, the duration of mating varies slightly with differences in the temperature of the oviposition room, the silkworm races, and number of matings accomplished by the male moths. Mating is a little shorter under high temperature. But for the silkworm races which have many unfertilized eggs or for the male moth to mate again, the time should be slightly lengthened. It is best for the male to mate once a day. If a second mating is desired of a male moth on the same day, the duration of the first mating should be reduced to three hours and the second increased to five hours. In mass egg deposition, it is common practice to duplicate the use of the male moths. If the mating is repeated on the same day, the male moths should be placed in cool rooms to rest for half an hour between the first and second matings.

The temperature during mating should be set at 24-25°C for bivoltine silkworms, at 27-28°C for multivoltines. The relative humidity in both instances should be maintained at 75-85 percent. Under high temperatures of about 30°C and dry conditions, the moths are apt to decouple. Moreover, the mating room should be quiet, uniformly lighted,

and free of drafts, and direct sunlight. When mating is in progress the rearer assigned to the job should make rounds of inspection. Whenever decoupling moths are found, they should be set elsewhere and left to mate anew.

Decoupling and moth broadcasting: When the time set for mating is nearly over, decoupling should begin. To decouple a mating pair, care must be taken not to apply force in pulling the moths apart, as the reproductive organs could be damaged. After decoupling, the mother moths should be shaken to accelerate their urination. Mother moths not having urinated or not having thoroughly urinated would not only have a late oviposition, but also would contaminate their eggs when they urinate in the course of oviposition.

It is advisable to collect the decoupled female moths on a piece of square soft cloth of 50 cm^2 on a side, or on a sheet of circular cloth of 50 cm in diameter. They should be dispersed evenly instead of in heaps. Then with two hands, the piece of cloth with moths on it should be lightly lifted and gently shaken up and down for three minutes. This will help the female moths to urinate thoroughly. Otherwise the moths will delay laying their eggs and the urine will spoil the kraft and contaminate the eggs.

The method of broadcasting the moths differs greatly with the method of egg laying employed. In loose formed egg laying, the number of female moths to be broadcast is correlated with the area of the ovulation cloth or kraft. The moths should be distributed evenly and allowed to oviposition without regard to how the eggs are laid or attached. But for the flat card egg deposition, on the other hand, uniformity in oviposition and attachment is expected. Neatness and pleasant appearance are other favourable aspects. For this reason, the number of female moths to be broadcast on every sheet is consistent, however, for the sake of accuracy and convenience, total weight is commonly used instead of number.

The following figures show the amount of female moths broadcast on a single kraft sheet in Guangdong Province: bivoltine eggs, 40-50 g; multivoltine eggs, 50-60 g. Finally, for the Pasteur's method eggs, only one mother moth is kept inside a galvanized iron ring. Deftness in handling is important.

Oviposition and round inspection: The diversity of oviposition conditions of silkworm moths is closely related to difference of race, time for mating, temperature during oviposition and strength of light. Comparing the oviposition speed of different races, the Chinese race is the fastest (generally, oviposition finishes at 21:00-22:00 hours the same day); the Japanese race is second; the European race is rather slow. The early eggs of all strains are larger than the late laid eggs. Also, egg laying is quicker when mating takes longer and will be slow if the mating time is short.

Oviposition can be accelerated if the temperature of the oviposition room is high and will be slowed down if the temperature is low. In addition, temperature affects the quality of the silkworm eggs.

At 24°C, fewer unfertilized eggs are laid, and at 26°C and 30°C, the opposite occurs. This is most conspicuous in the Japanese strain.

As to the speed of oviposition, at the outset it is a little fast at 26.5°C and 30°C and will slow down four hours later. Eight hours later, the rate of eggs laid surpasses 95 percent. Usually the rate of oviposition reaches approximately 95 percent eight hours after decoupling.

About two hours after oviposition, the nucleus of the egg fuses with that of the sperm cell to form a zygote, and fertilization is achieved. In succession, the zygote undergoes cell division. It is at this stage that the silkworm egg has the lowest resistance to an adverse environment. If the temperature should rise to 30°C and above, it is apt to produce unfertilized or even dead eggs.

The egg laying room should be maintained at its optimum humidity of 75 percent. Moreover, dark chambers are favourable for rapid oviposition, while bright chambers have the opposite effect. The doors and windows of these chambers should be shaded with black curtains. Red light is preferred for round inspection. Round inspection should be done once every two hours, especially at 16:00-17:00 hours, when oviposition is most vigorous.

Storage management and remating of the male moths: On observing the patterns of emergence, one notices that the emergence of male and female moths is not balanced. In the common commercial race, emergence in the early stage produces more males than females and the reverse in the final emergence (exceptions exist in some rare strains). Thus, the extra males in the early stage must be adequately preserved for further use in case there are not enough males for coupling. Also, it is customary to preserve the males after a first mating in order to remate them again when necessary.

Storage management of male moths: It is necessary to maintain the vigour of the male moths under storage. Generally, they can be preserved at a low temperature of 5-7.5°C for four or five days, but any longer will cause the mating capability to be subdued and the number of unfertilized eggs to increase. The male moths should not be piled up in the container for storage, but laid in one layer. To avoid confusion, they should be indexed with name of the race, serial number of the batch, date of emergence, and the number of matings.

Remating of male moths: The remating of the male moths is a common practice in production. Based on investigations of the mating capability, one male moth can mate eight females under optimum utilization and preservation conditions. But as the number of matings increases, the number of unfertilized eggs will also increase. This is quite obvious when the male moths have mated three or more times. Therefore, it is necessary to avoid more than three matings in production.

Cold storing of female moths: The chilling of female moths, during production, should be avoided as much as possible. Based on experiments, the oviposition function will be completely inhibited at 2.5°C. Even if the temperature is raised to about 5°C, there is still danger of oviposition during chilling. Hence, cold storing at 5°C is near optimum. But this is limited to only three days, and unfertilized eggs are produced after the fourth day. The chilling of the female moths should be carried out right after emergence when their bodies have become dry and fresh and their wings fully stretched, otherwise oviposition will occur during chilling. The possibility to lay eggs during chilling is related to the different characteristics of the races, the Chinese silkworm race being most likely, the Japanese race next and the European race least likely.

Moth collecting and preservation of mother moths: Most of the silkworm eggs are laid in the same evening before 22:00 hours, but the process continues until about midnight, when oviposition ceases and the moths should be collected. However, since the eggs are expected to hatch at different times, moth collecting may proceed or be delayed accordingly. If the silkworm eggs are used for artificial hatching treatment, the mother moths must be collected at 21:00-22:00 hours in the same evening. If, however, they are intended for hibernating eggs, there is no harm in postponing moth collection until the following morning. It must, however, be kept in mind that the later the eggs are laid, the more unfertilized eggs are produced.

During moth collection for production F_1 hybrid eggs, 10 percent of the mother moths are taken out and put into boxes, indicating the race, date, batch and serial numbers (Figure 5-5).

After moth collection has been completed, the boxes are bound according to races, dates, batch numbers and serial numbers and are stored in well ventilated dry places. Care should be taken in not allowing the boxes to pile in heaps, to avoid them giving out heat and being damaged by moulds. Drying is done after the moths die naturally in order to permit the pebrine spores to multiply to the maximum; this facilitates microscopic inspection. The temperature for drying the mother moths should be maintained at 60-65°C for four to five hours at the most. If the temperature increases to 90°C, the pebrine spores are liable to deform and affect microscopic inspection. The dried moths can be inspected any time. The moths should be well protected against insect or rat attacks or mould.

Figure 5-5
Moth box

Harvesting of silkworm eggs: The harvesting of silkworm eggs is carried out after moth collection. Moths should be collected systematically in conformation to the race, batch number and serial numbers. Egg cards are inserted into wired frames or hung on wires with no piling or heaping to avoid disturbances of the physiological soundness of the eggs. At this time, the hibernating eggs should be protected from high temperatures so as to stabilize their hibernating characters. For eggs intended for artificial hatching, preparations either for acid treatment or for storage should be made well ahead of time. Eggs to be transported must be inserted into wired frames and transported in the early morning or after nightfall.

CHAPTER 6

EXAMINATION FOR THE PREVENTION OF PEBRINE DISEASE

6.1 Importance of Examination for the Prevention of Pebrine Disease

Pebrine disease in *Bombyx mori* is caused by the parasitic spore of *Nosema bombycis* of the family *Nosematidae* and is transmitted through oral or maternal infections to the offspring. The disease causes great damage to the silk industry. An epidemic of pebrine nearly wiped out sericulture in France, Italy and other countries. Only after Louis Pasteur, a French scientist, discovered the route of the pebrine infection and created the method of examining the mother moth for signs of pebrine, could the disease be controlled.

The so-called disease-free eggs means the silkworm eggs do not carry the spore of *Nosema bombycis*. For the purpose of securing disease-free eggs and avoiding losses in production, measures for checking the quality of egg breeding and multiplication have been worked out in many sericultural countries. Predictive inspection for pebrine and the qualified standard for examination of the mother moths are described in this Chapter. For example, in East China the standard for the rate of pebrine permitted in the whole lot of parent eggs is below 0.1 percent and for the F_1 hybrid, below 0.2 percent.

6.2 Methods of Examination of Pebrine Disease

Supplementary examination: The supplementary examination is usually made to ensure that the parent silkworms, grandparent silkworms, and newly introduced silkworms are free from the pebrine. Sometimes, it is used to correct oversights or errors in the examination of mother moths for pebrine disease. The process is as follows:

Cardboards are prepared for the attachment of test materials (about half the size of the egg card). 20-50 defective eggs are picked out from each egg batch before incubation. They are stuck onto the cardboard for supplementary examination, the identifying numbers recorded, and treated with earlier incubation. After hatching, the newly hatched larvae are allowed to starve to death naturally under an environmental condition of 30°C and a humidity of 85 percent. The dead larvae are pound together with their egg shell (still keeping track of their corresponding numbers) and subjecting them to microscopic examination. If any diseased egg batch is discovered, this should be burnt.

Examination of late-moulting worms in different instars: In the rearing of parent silkworms, attention should be paid to the examination of the silkworms in different instars. The late moulting worms and the underdeveloped worms should be put into different bags according to their different egg batches, their batched numbers annotated and are then stored in heat regulators. After preservation at

29-30°C under humdity of 90-95 percent for two days, the larvae should be examined under a microscope.

If pebrine spores are detected, the rearing batch should be discarded. Since the body size in grown larva is big, it is more convenient to examine its alimentary canal. If pebrine spores are detected in the fifth instar, these silkworms may be used for reeling cocoon rearing according to the degree of infection. However, these silkworms should be separated and thoroughly disinfected.

Accelerating the emergence of the moths for examination: The pathogen of pebrine disease in silkworms is easily examined at the moth stage, for by then it has fully developed into a spore. The result is always satisfactory. The process is as follows:

One hundred early mature larvae are chosen from different batches, put on a separate mountage to spin cocoons and preserved at 30°C under humidity 80-85 percent in order to accelerate their pupation. The emergence of the moths will be two to four days earlier than the rest. The early emerged moths should then be examined under the microscope. If the percentage of pebrine infection is higher than what is permitted, the entire lot of seed cocoons should be dried out and destroyed. The cocoons can, however, still be sold for silk reeling.

Examination of the mother moths: Examination of the mother moths is an important technical measure for the prevention of pebrine disease transmitted from the mother moths' body to the offspring. In order to ensure the quality of examination, careful preparation should be made as follows.

Regulations for boxing the mother moths: All the parent moths should be boxed according to their serial numbers and the number of the egg cards. Five to ten percent of F_1 hybrids are taken as samples and boxed according to the number of egg cards. The moths laying eggs last are called the last moths; all of them should be boxed.

Regulations for the microscopic examination of the mother moths: For the original strain of authorized race and grandparent race, each mother moth should be examined twice by two persons. For the parent mother moths, 20 percent of each batch should be examined twice by two persons at first. If no pebrine spore is found, the rest of the moths can be examined by only one person. For F_1 hybrids, half of the boxed moths can be examined first. If no pebrine is found, the rest can be left without being examined. If pebrine spore is found, the rest should be thoroughly checked. The infection rate is calculated by taking the average. If the infective rate is over what has been permitted, the entire lot of silkworm eggs should be burned.

Labour organization: The personnel is divided into two groups, one group for the management of inspection, and the other for microscopic examination.

The specific tasks for the management group are: sampling, distributing, registering the material for microscopic examination, and filling in and checking the examination forms. The number of persons assigned to this group is determined according to the workload.

In the microscopic examination group, five to seven persons are needed to do the first examination, and one person for re-examination, four to five persons for grinding the moths and dipping the moth juice on the slides, two persons for washing the slides, pestles and mortars.

Examination processes

Taking the moths from the boxes: This is done according to the serial number of the boxes. The label marked with the lot number of the moths is torn off and is put at one end of the mortar; the box is then opened and with the use of forceps the moths put into the mortar.

Grinding the moths: When grinding the moths, 1 ml of 2 percent KOH solution is poured onto each moth. One pestle is used for each moth. The moths must be ground into fine and broken bits.

Dipping the moth juice onto the slide: A label is clipped onto the slide holder, and the slides arranged on the holder. The moth juice is dropped onto the slide according to its corresponding number. While dropping, the pestle should touch the juice to the bottom. The optimum size of drop is about 0.6-0.7 cm in diameter. The juice is in moderate density and should not contain any other foreign matters. After dropping, it should be covered with a cover slip and sent to the first examiner (Figure 6-1).

If pebrine spores or other suspects are found in the first examination, it should then be sent to the second examiner. If no pebrine spore is found, the slides should be merged with pebrine-free juice on to one slide and then sent to the second examiner.

Figure 6-1
Mortars, pestles, and slide holder

Microscopic examination: In the first examination, a microscope with 600-800 magnification is used. Three-five fields of a microscope are taken for each sample and the results of the examination are recorded on an examination form. If pebrine spore is detected, the slide should be submerged in a disinfectant solution.

In re-examination, the examiner should check all the samples and then mix them up. One or two samples should be dropped onto the slide and more than five fields of a microscope should be taken for each sample. If pebrine spores are detected, the first examiner should be informed. He should drop the juice of each moth onto the slide and re-examined each slide individually. If no spores are found, the slides should be washed.

Washing: The pestles, mortars, slides and cover slips should be washed separately in clean water. The appliances contaminated with diseased moths should be submerged in a strong hydrochloric acid solution, disinfected for 15 minutes and then washed in clean water.

Straightening up the laboratory: At the end of each working day, the remaining moth boxes and the examined moth boxes should be checked and the the daily record forms completed. All discarded boxes should be burnt and all appliances thoroughly disinfected and put in order.

Calculating the percentage of pebrine disease: After microscopic examination of each lot of silkworm eggs, the percentage of the diseased moths should be calculated as follows:

$$\text{Diseased moths percentage (\%)} = \frac{\text{Number of infected moths}}{\text{Number of moths examined.}} \times 100$$

CHAPTER 7

PRESERVATION OF SILKWORM EGGS

7.1 Importance of Silkworm Egg Preservation

Preservation refers to the management and protection of the eggs during the period from the laying to hatching of the hibernating eggs. Not only is it a long time from egg laying to hatching, but the hibernating egg laid in spring must endure the hot summer and cold winter. Although the hibernating eggs are mostly at diapause, their physiological activities, such as respiration and metabolism, are still going on. The changes of external conditions have direct influence on the dediapausing process of the eggs. Proper preservation conditions must be maintained in accordance with the physiological requirement and development of the silkworm eggs, and special care must be taken by an assigned person. If preservation is not done properly, more eggs will die, hatching will not be uniform, the larvae of the next generation will be weak and the sericultural production will be affected, or in extreme cases, lose its entire value.

Summer is especially long in the tropical and sub-tropical areas, and both humidity and temperature are high. But in winter, the low temperature period is short, and natural conditions are not sufficient to awaken the hibernating eggs from diapause. Particular measures must, therefore, be taken to preserve the eggs in these regions. A broad knowledge on the developmental stages of the embryo is essential for the preservation control.

7.2 Embryonic Development

Pre-embryonic developmental stage

Division of the zygote nucleus: The spermatozoon enters the ovum to form a male pronucleus, then fuses with the female pronucleus to form a zygote, thus completing fertilization. The zygote nucleus then undergoes cell division, dividing into two cleavage nuclei. The two cleavage nuclei continue to divide and produce many daughter nuclei (called cleavage energids). Distributed in the yolk of the egg, these gradually migrate to the periphery of the egg.

Formation of the blastoderm: The cleavage energids in the periphery of the egg gather themselves under the periplasm and form a cell layer of uniform thickness. This cell layer is called the blastoderm.

The formation of germ band and serosa: When the blastoderm has been formed, the layer of blastoderm cells at the micropyle's side becomes densely packed and gradually thickens. This thickened cell layer is called the germ band. However, the cell layer on the opposite

side of the micropyle expands and becomes gradually thinner, forming a thin membrane called the serosa.

Formation of the amnion: After the germ band is formed, its periphery sinks into the egg. The serosa linking the periphery of the germ band rises and folds appear. These folds, called amniotic folds, stretch along the sides of the germ and finally fuse with each other. The fold edges disappear to become two separate layers of membranes. The outer membrane, the serosa, closely adheres to the lower surface of the vitelline membrane. The inner membrane is the amnion covering the ventral side of the germ band. Between the amnion and the germ band there is an empty space called the amniotic cavity; this is filled with fluid for the protection of the germ band. After separating from the serosa, the germ band develops into an independent embryo. The embryo connects with the amnion and continues to develop inside the yolk.

The principal stages of embryonic development: Embryonic development can be divided into 15 stages, knowledge of which is essential for preservation of silkworm eggs, cold storage, artificial hatching, and incubation, and also for the control of voltinism (Figure 7-1).

7.3 Methods of Preserving the Silkworm Eggs

Preservation of eggs produced in spring until the following spring: These eggs are produced in spring and preserved for rearing in the following spring. In the sub-tropical regions, the production of the eggs is carried out at the beginning or in the middle of April, and these eggs should be preserved for rearing in the middle or at the end of February of the following year. Preservation should last as long as ten months. This is difficult to achieve, however, since in the sub-tropical regions the low temperature conditions needed to awaken the embryo from diapause are lacking or inadequate. However, modern science and technology have made it possible to control environmental conditions artificially.

Early stage preservation of the eggs (about ten days after laying): After the eggs are laid, the nuclei of the male and female fuse to form a zygote, which in turn begins to divide and gradually develops into an embryo. In about a week, the embryo enters a state of diapause. The egg is light yellow when first laid, it gradually changes into reddish brown after one and a half to two days, and the colour continues to darken. On the fifth day, the egg acquires its final colour. During this period, respiration is strong as a result of the rapid development of the egg. Care should be taken not to shock, crush, or rub the eggs. To avoid irritating odours coming into contact with the eggs, the egg card should be hung in well-ventilated, clean rooms at a constant temperature of 24°C and a relative humidity of 75 percent. High temperature and high humidity must be avoided, for it not only inhibits the physiological activities of the eggs but also results in abnormally hatched eggs.

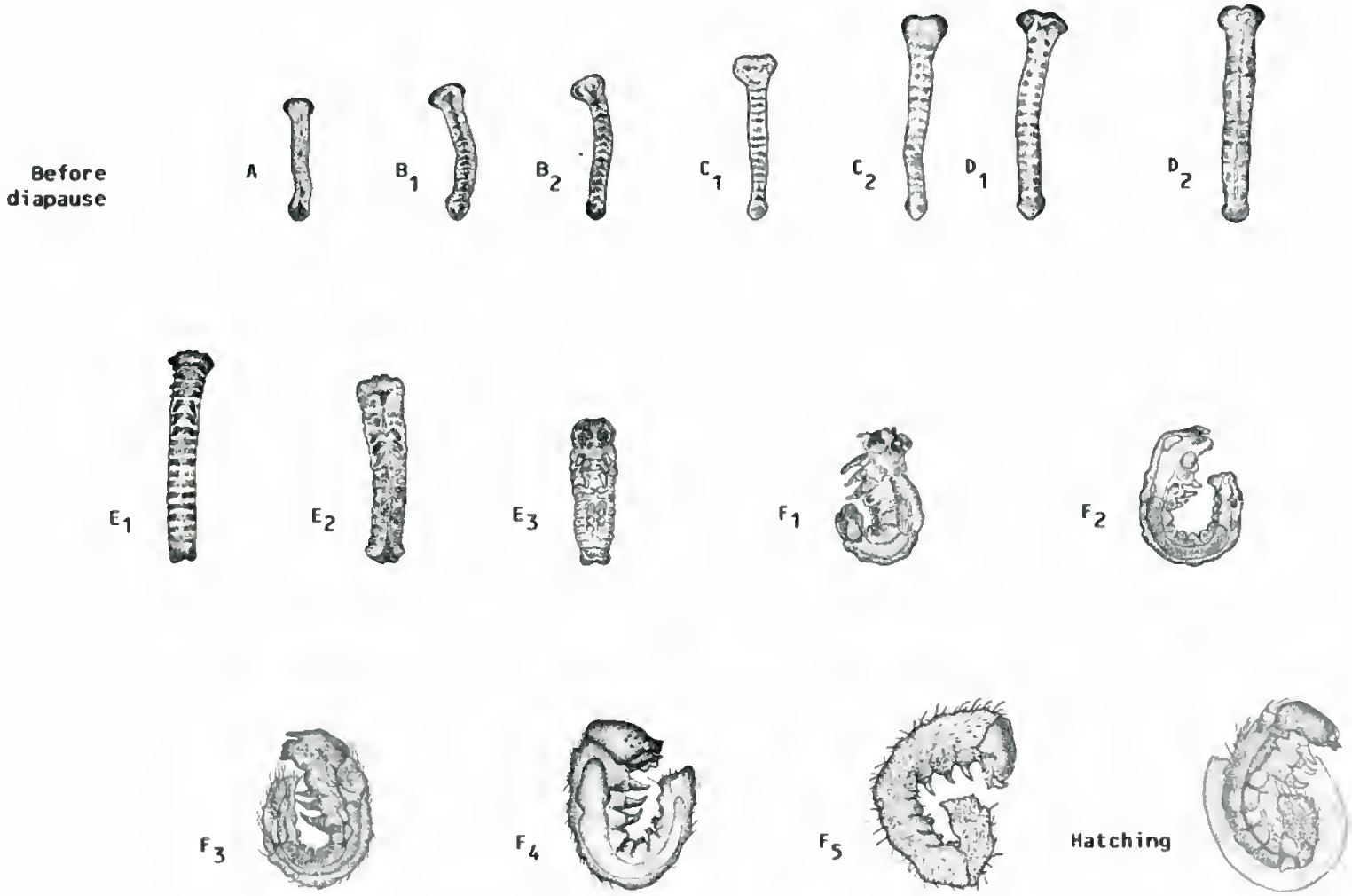


Fig. 7-1 Principal developmental stages of the embryo and its features.

Preservation in summer: The spell of high temperature in summer, through which eggs produced in spring for rearing in the next spring have to pass, is a requisite for stabilizing the diapause of the hibernating eggs. However, the temperature should be sufficiently high to permit aestivation: experience has shown that 25°C is the safest. Temperatures above 30°C are very harmful to the physiological activities of the eggs and the longer the eggs are exposed to high temperature, the more they will suffer. Temperatures below 20°C may disturb the diapause of the eggs, making the hibernating eggs unable to withstand cold storage, and may disturb uniformity in embryonic development.

As to the duration of preservation, no inhibition to diapause occurs when the eggs are kept at 25°C for 90 days. The longer the preservation, the deeper the degree of diapause. This diapause is broken more slowing and the eggs will withstand cold storage longer. If preservation lasts longer than 90 days, the time the eggs can endure cold storage will be shorter, and the hatching of the eggs will be unsatisfactory. For safety in production, it is advisable to preserve the eggs no more than 60 days at 25°C in summer.

The preservation period for univoltine races, the Japanese, and European races can be a little longer, but for the bivoltine races and the Chinese races, it must be a little shorter.

The optimum humidity for summer preservation is 75-80 percent. If the air is too dry, much of the eggs' water will be lost and their physiological activities will be affected. If it is too wet, moulds are apt to grow on the eggs. Furthermore, air must be fresh in the preservation room. Timely ventilating is necessary according to the weather conditions.

In the tropical and sub-tropical areas, these requirements can be met only when the silkworm egg preservation rooms are air-conditioned.

Preservation in autumn: Hibernating eggs produced in spring, after summer preservation at high temperatures, should be kept at lower temperatures in order to meet the physiological requirement of the developing eggs. The optimum range is 22°-20°C, with the temperature higher in the earlier period and lower in the later period. The relative humidity should be about 80 percent. Under such environmental conditions, preservation can last as long as two to three months. In some areas, as the weather in autumn is dry, it is necessary to increase the humidity. Care however must be taken to ventilate the rooms to prevent the eggs from becoming mouldy.

Preservation in winter: This subject will be dealt with under cold storage of hibernating eggs.

Preservation of autumn eggs until the following spring: Silkworm eggs produced in Autumn for rearing in the following Spring are called "eggs produced in Autumn and hatched in the following Spring". As preservation lasts a shorter time, less of the nutrients in the eggs

are consumed and it is easy to maintain the quality of the eggs. For production of hibernating eggs in the tropical and sub-tropical areas in particular, it is best to produce in autumn. Unless absolutely necessary, it is advisable not to produce eggs in spring for rearing in the following spring.

The preservation of autumn eggs for the following spring is the same as for the spring eggs for the following spring in the early stage (about 10 days after laying).

Artifical aestivation for the hibernating eggs is produced in autumn. In some areas, after the production of eggs in autumn, the natural temperature is relatively low. If the room temperature is below 22°C, the eggs will be in a low degree of diapause, the embryo will be awakened too early and the eggs will not endure cold storage. Therefore it is necessary to employ artificial aestivation. This is to preserve the eggs produced in autumn at a temperature of 25°C and a relative humidity of 80 percent for about ten days when the eggs have attained their inherent colour. Timely ventilation is necessary, and the position of the eggs should be changed from time to time to ensure uniform temperature-sensitivity.

In some areas, after the production of eggs in autumn, if the natural temperature is still high, care must be taken to protect the eggs by lowering the temperature to 24°-25°C, avoiding temperatures higher than 27°C and maintaining a relative humidity of 80 percent. Otherwise white dead eggs are likely to appear.

How long should the autumn eggs be preserved at a high temperature? It depends on when they are to be used the following spring. If they are taken out earlier for incubation, the temperature should be lowered earlier, and the eggs should be transferred to cold storage earlier. If they are taken out later, the temperature may be lowered a little later, and the eggs are transferred to cold storage later.

This is because once the silkworm eggs enter diapause, they must be preserved at low temperatures as long as 120 days to break the diapause. This is a conservative characteristic of the hibernating eggs formed in the course of systematic development.

Therefore, while considering the time for high temperature preservation of the autumn eggs, it is necessary to consider the time needed to break the diapause of the eggs and when the eggs will be used in the coming year. For example, if the eggs are prepared in early October and used in early March of the following year, the time for high temperature preservation can be decided thus:

The time from egg production to incubation is five months (October to March). The time for low temperature preservation to break the diapause is four months. There is, therefore, still one month from

egg laying to transferring the eggs into cold storage. During this month, the eggs can be treated as follows:

- Preserved at 25°C for 20 days after laying.
- The temperature lowered in stages in the following ten days. For the first five days it is kept at 20°C and at 15°C for the next five days.
- After preserving in this way for a month, the eggs are kept in cold storage for hibernation.

Washing of the silkworm eggs and disinfection of the egg surface: In order to get rid of the scaly hair, urine and pathogenetic micro-organisms attached to the surface of the silkworm eggs, the eggs must be washed and disinfected. This procedure can be done two weeks after oviposition or just before transferring the eggs into cold storage. The practical method is to immerse the silk eggs in a 2.5 percent diluted formaldehyde solution under normal room temperature for 40 minutes, turning the egg cards up and down once during immersion. After disinfection, the eggs are transferred into clean water. In washing, a broad brush consisting of a row of Chinese pen brushes is used to brush the egg surface card by card. The work should be finished in an hour, and the egg cards should be hung up individually to dry.

7.4 Cold Storage of Hibernating Silkworm Eggs

Aim of chilling the hibernating silkworm eggs: The hibernating silkworm eggs must endure low temperatures before they begin to develop and grow. This is a genetic character formed during the systematic development. In temperate areas, the natural winter temperature is low enough to break the diapause of the hibernating eggs. When winter changes into spring, the temperature changes greatly and the embryonic development of the dediapaused silkworm eggs is not uniform. Some eggs even hatch too early while the mulberry trees are still in bud. In order to set an accurate date for incubation and collection of the newly hatched larvae as planned, the eggs should be transferred into cold storage in late winter for preservation. Eggs can be taken out of cold storage for incubation according to the plan. In sub-tropical and especially tropical areas, it is necessary to transfer the silkworm eggs into cold storage earlier to simulate hibernation for the purpose of awakening the embryo from diapause.

Thus it can be seen that the cold storage of the silkworm eggs in the earlier stage has the function of breaking the diapause, and in the later stage, it plays the role of inhibiting the embryonic development of the silkworm eggs. The purpose of cold storage of silkworm eggs is to avoid non-uniform development of the embryos and too-early hatching of the eggs and to control the date for brushing the newly hatched larvae.

The embryo of the hibernating egg and the optimum temperature of cold storage: The time required for the completely dediapaused embryo to develop from A stage to C_2 stage varies, depending on race and season of egg deposition. Chinese and Japanese bivoltine races develop comparatively faster; Chinese and Japanese univoltines, the second; and European univoltines are the slowest.

Embryos from A stage to C_2 stage have a strong resistance to low temperature. In fact, the younger the embryo, the stronger its resistance to low temperature. The optimum temperatures for cold storage of embryos at different stages after hibernation are as follows:

A embryo -2.5°C; B_1-C_1 embryo 0°C; C_2 embryo 0-2.5°C. After C_2 stage, resistance to low temperature weakens sharply. At present, the temperatures adopted for cold storage of hibernating eggs are: A embryo 0-2.5°C; C_1-C_2 embryo 2.5°C.

Methods of chilling the silkworm eggs: There are two methods of chilling the hibernating silkworm eggs: single-step cold storage and two-step cold storage.

Single-step cold storage: This is a commonly used method, it may vary with climatic conditions in different areas. In temperate areas the natural temperature in winter meets the physiological requirements of the silkworm eggs for hibernation. Therefore, the silkworm eggs can be preserved under normal room temperature. Then, in middle or late January, from C_1 the period before the longest embryo stage (C_2), the silkworm eggs should be transferred to and kept in cold storage at 2.5°C until mid-April. The date for releasing the eggs from cold storage for incubation will depend on the leaf-shooting conditions of the mulberry trees.

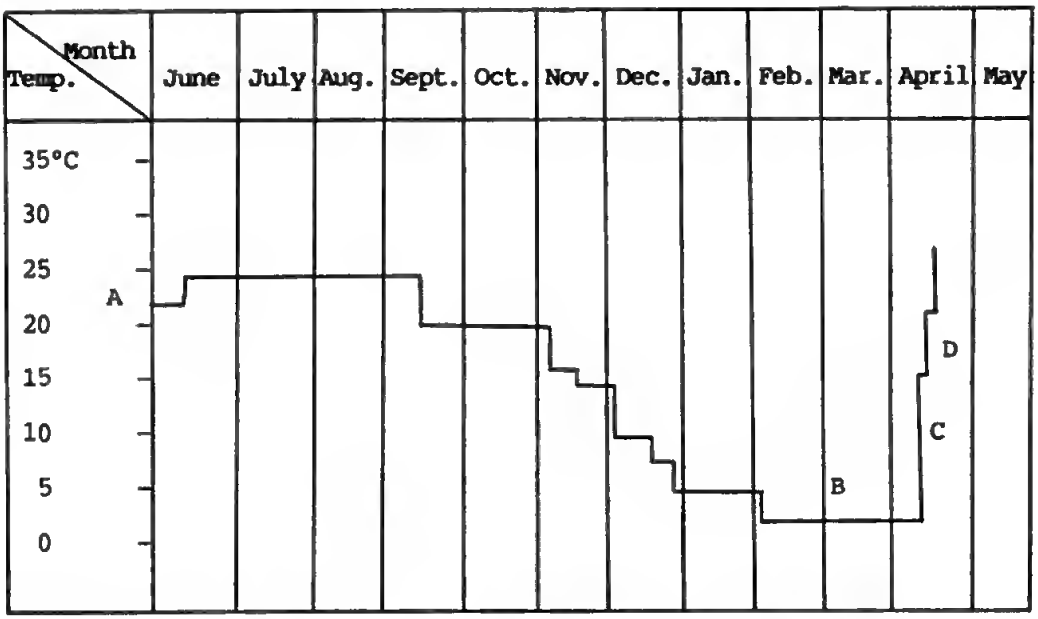
Figure 7-2 shows the criterion for preservation and single-step cold storage of the hibernating eggs produced in spring in temperate areas.

In tropical and sub-tropical areas, not only is the winter temperature too warm to break the diapause, but incubation begins early in spring. Therefore, cold storage should begin at an earlier date. The eggs should be preserved at 2.5°C for 120 days. Should preservation last less than 120 days but more than 100 days, the eggs can be taken out of cold storage and subjected to acid treatment, and a good hatching can still be achieved. If chilling exceeds 120 days but is less than 150 days, no evident effect appears on the hatchability of the silkworm eggs. But if the cold storage period is prolonged even further, hatchability will gradually decrease.

Table 7-1 shows the criterion for the preservation of the hibernating eggs produced in spring in South China.

Two-step cold storage: Sometimes, silkworm eggs should undergo a rather long period of cold storage, in which case a two-step cold storage procedure is required. This means that silkworm eggs undergo cold storage twice in their two different embryonic development stages, and the cold storage can be prolonged. The method of two-step cold storage is the following.

Step one consists of eggs being delivered to cold storage in their A embryo stage. The cold storage temperature in temperate areas is 2.5°C, in tropical and sub-tropical areas it is 5°C. Time limit for cold storage is 90-100 days.



Note: A = Egg production C = Released from cold storage
B = Enter cold storage D = Incubation

Figure 7-2
Criterion for the preservation and single-step cold storage of hibernating eggs produced in spring in temperate areas

Table 7-1

Criteria for the preservation of hibernating eggs produced in spring in South China (Chinese bivoltine x Japanese bivoltine; eggs are laid in the middle of April)

Preservation period (day/month)	Duration (days)	Temperature (°C)	Difference between wet and dry bulbs of psychrometer (°C)	Effective accumulated temperature (°C)
20 Apr.-20 Jun.	61	25.5	1.7	945.5
21 Jun.-31 Aug.	72	22-23	1.7	828.0
1-30 Sept.	30	18	1.0	240.0
1-15 Oct.	15	15	1.0	45.0
16 Oct.-12 Dec.	120	2.5		

Step two consists of taking the silkworm eggs out of cold storage when they have reached the time limit for the first stage and further preserved for four to five days at 15°C and a relative humidity of 85 percent. The development of the silkworm eggs should be observed by dissecting. When the silkworm eggs reach the C_1-C_2 embryonic development stage, they should be returned to cold storage; the temperature required is 2.5°C. Although the limit for the second stage may be as long as 100 days, it is safer not to exceed 80 days.

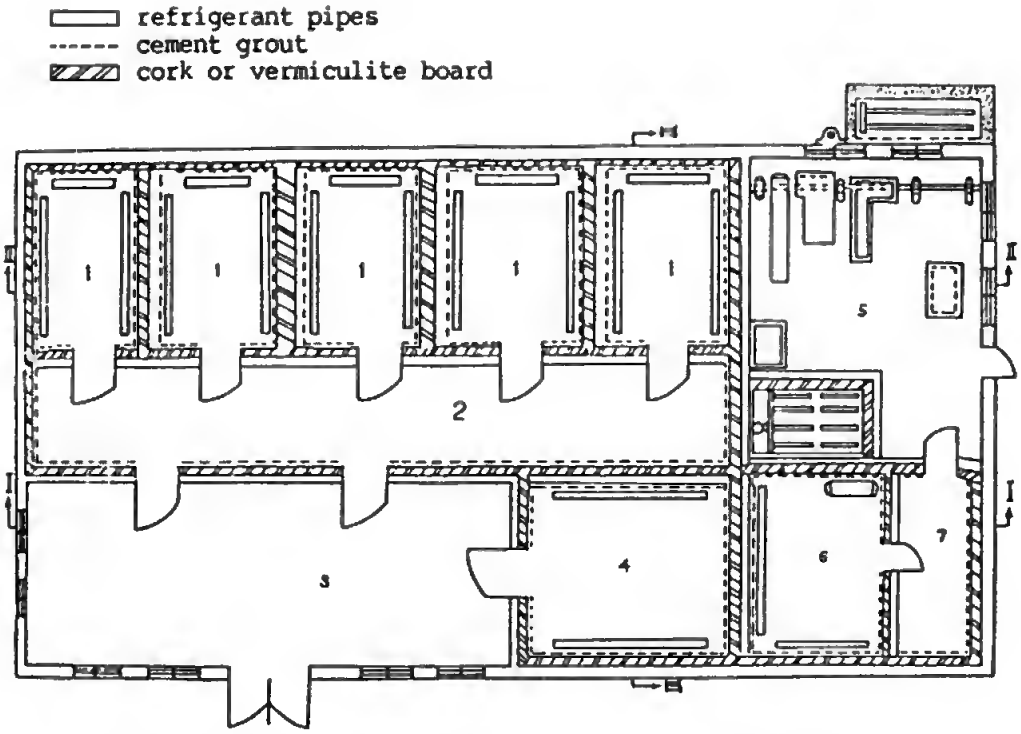
7.5 Cold Storage Installations

Structure of cold storage: There are two types of cold storages: direct cooling and indirect cooling. In direct cooling, refrigerant pipes stretch into the cold storage and cool the air there directly. In indirect cooling, salt solution is cooled by refrigerants first, and then the refrigerated salt solution is piped into the cold storage to cool the air inside. The salt solution may be either sodium chloride or a calcium chloride solution of 20 percent concentration. The freezing point of the sodium chloride solution is -17°C and that of the calcium chloride solution is -23°C. Indirect cooling is safer for the silkworm eggs.

Inside the cold storage, the walls, the flat roof, the floor, and the door should have a layer of insulating material about 15 cm thick. The insulating layer of the main storage room is about 10 cm thick. Of all the insulting materials, cork board is preferable, and its thickness should be increased properly if sawdust or rice husk is used instead. The outer part of the insulating material should be covered with asphalt felt and galvanized wire fabric and plastered with cement grout about 2 cm thick. Rubber strips are stuck in the door cracks to prevent the cold air from escaping.

The main equipment of the cold storage include compressors, motors, diesel engines, condensation towers, salt solution tanks and pumps, liquified ammonia cylinders, and automatic control devices.

The construction of the cold storage installation has the following parts: the silkworm egg cold storage rooms, the vestibule room, the low-temperature room, the egg-card arrangement room, and the engine room (Figure 7-3).



1. Cold Storage room
2. Vestibule room
3. Egg-card arrangement room
4. Low-temperature room
5. Engine room
6. Ice storage
7. Vestibule room for ice storage

Figure 7-3
Cold storage (design drawing)

7.6 Substances Harmful to Silkworm Eggs

There are many substances harmful to silkworm eggs. The most likely to be encountered in the handling and preservation of silkworm eggs are: tobacco, agricultural chemicals, oils, mercury, paste, acid and alkaline substances, and peculiar odours or bad smells.

Furthermore, there are other natural enemies, such as wild rats, insect pests and moulds, and contact with these should be strictly prevented.

CHAPTER 8

ARTIFICIAL HATCHING OF SILKWORM EGGS

Under normal conditions, hibernating silkworm eggs remain in diapause for a year after being laid and will not hatch regardless of temperature. Only after overwintering and being exposed to low-temperature for a sufficient period can they develop and hatch in the coming warm spring. Therefore, silk production will be restricted to a certain degree. As early as the nineteenth century, China and some European countries had mastered the art of artificial hatching of silkworm eggs. That is to say, they had succeeded in breaking the diapause of the hibernating silkworm eggs by applying artificial physical, or chemical stimulants at a certain stage of the embryonic development, so that the eggs hatch within the year. This can be repeated several times within one year.

8.1 Methods of Artificial Hatching

The methods of artificial hatching can be divided into two main groups, physical and chemical.

Physical methods:

- artificial overwintering
- warm-water treatment
- stimulation by friction
- stimulation by high temperature
- stimulation by low-temperature
- stimulation by high atmospheric pressure
- exposure to sunshine
- electric induction.

Chemical methods:

- hydrochloric acid treatment (common acid treatment and acid treatment after cold storage)
- nitric acid treatment
- sulphuric acid treatment
- aqua regia treatment
- sodium chloride solution treatment

An artificial hatching method should have/be:

- a high hatching rate
- a uniform hatching rate
- simple equipment
- easy manipulation
- high work efficiency
- economical in labour and cost

8.2 Hydrochloric Acid used for Artificial Hatching

Pure hydrochloric acid would be safe, but quite expensive. Industrial hydrochloric acid is used in artificial hatching, but it should be tested before use. The saturated solution of pure hydrochloric acid contains 42.09 percent hydrogen chloride at a temperature of 15°C; the specific gravity is 1.212; it is colourless and transparent, dissolves easily in water, vaporizes immediately into a thick white "fog" when it comes into contact with the air; it is a strong stimulant and corrosive. Industrial hydrochloric acid contains 30 percent hydrogen chloride; its specific gravity is 1.160; and it is light brown or yellow in colour. Hydrochloric acid has a strong disinfecting activity. Thus, acid treatment has the additional effect of disinfecting the egg surface. With heated acid, the muscardine spores of the silkworm eggs could be killed in three seconds.

The specific gravity of the hydrochloric acid is in direct ratio with its concentration. The greater the specific gravity, the higher the concentration (Table 8-1). The specific gravity of hydrochloric acid varies with the temperature of the acid solution. The higher the temperature of the acid solution, the lighter the specific gravity. Above 15°C, when the temperature increases or decreases 1°C, the coefficient of the specific gravity of the hydrochloric acid varies as follows:

Range of specific gravity	Variation of coefficient of specific gravity
1.065-1.075	0.0003
1.080-1.100	0.0004
1.105-1.120	0.0005

The approximate specific gravity of the hydrochloric acid can be estimated as the temperature changes by the variable coefficients of the specific gravity of the hydrochloric acid. For example, at 24°C, the specific gravity of the hydrochloric acid is 1.075. When the temperature increases to 46°C, the specific gravity is:

$$1.075 - (46-24) \times 0.0003 = 1.0684$$

Another example: at 47.8°C, the specific gravity of the hydrochloric acid is 1.092. When the temperature decreases to 24°C, the specific gravity is:

$$1.092 + (47.8-24) \times 0.0004 = 1.1015$$

Table 8-1

Relationship between specific gravity and concentration of hydraulic acid

Specific gravity (d^{15}_{4}) in vacuum	Baumé Degree	Concentration (%)	Specific gravity (d^{15}_{4}) in vacuum	Baumé Degree	Concentration (%)
1.000	0.0	0.16	1.115	14.9	22.86
1.005	0.7	1.15	1.120	15.4	23.82
1.010	1.4	2.14	1.125	16.0	24.78
1.015	2.1	3.12	1.130	16.5	25.75
1.020	2.7	4.13	1.135	17.1	26.70
1.025	3.4	5.15	1.140	17.7	27.66
1.030	4.1	6.15	1.143	18.0	28.14
1.035	4.7	7.15	1.145	18.3	28.61
1.040	5.4	8.16	1.150	18.8	29.57
1.045	6.0	9.16	1.152	19.0	29.97
1.050	6.7	10.17	1.155	19.3	30.55
1.055	7.4	11.18	1.160	19.8	31.52
1.060	8.0	12.19	1.163	20.0	32.10
1.065	8.7	13.19	1.165	20.3	32.49
1.070	9.4	14.17	1.170	20.9	33.46
1.075	10.0	15.16	1.171	21.0	33.65
1.080	10.6	16.15	1.175	21.4	34.42
1.085	11.2	17.13	1.180	22.0	35.39
1.090	11.9	18.11	1.185	22.5	36.31
1.095	12.4	19.06	1.190	23.0	37.23
1.100	13.0	20.01	1.195	23.5	38.16
1.105	13.6	20.97	1.200	24.0	39.11
1.110	14.2	21.92			

Diluting the hydrochloric acid: There are two methods of diluting the original solution of hydrochloric acid to an objective concentration for acid treatment of silkworm eggs.

(1) Dilution based on specific gravity:

$$\text{amount of original solution in the bulk of dilute acid (\%)} = \frac{A - 1}{B - 1} \times 100$$

$$\text{multiple of water added} = \frac{(B - 1) - (A - 1)}{A - 1} \times 100$$

A = objective specific gravity
B = specific gravity of the original solution

(2) Dilution based on concentration:

$$\text{multiple of water added} = \frac{a\ (\%) - b\ (\%)}{b\ (\%)}$$

a = concentration of the original solution
b = objective concentration

8.3 Artificial Hatching by Common Acid Treatment

Artificial hatching by common acid treatment demands an immediate hydrochloric acid stimulant right after the egg is laid, and before the appearance of hibernating characters. This will block the hibernating tendency and render the embryo ability to continue development into hatching instead of entering diapause. There are two possible methods: heated acid treatment and acid treatment at normal room temperature.

Heated acid treatment

Appropriate time for acid treatment: It is particularly important to conduct acid treatment at the appropriate time. The best time is between the fusion of the amnion fold and the formation of an independent embryo. Generally, this is 16-30 hours after the eggs are laid. However, if the preservation temperature after egg laying is high, acid treatment should be done a little earlier, and a little later if the preservation temperature is low (Table 8-2).

Table 8-2

Optimum time for common acid treatment of bivoltine eggs

Preservation temperature (°C)	Time after eggs are laid (hours)	Accumulated effective temperature (°C)
21	25-35	275-385
24	18-26	252-364
27	16-23	272-391
29	16-23	

Time is calculated from the vigorous stage of oviposition when the mother moths lay 50-60 percent of their eggs.

When the preservation temperature after the eggs are laid is between 27-30°C, there is no harmful effect on egg development. A preservation temperature above 30°C is harmful to the silkworm eggs, and the development of the embryos slackens as a result.

Concentration of hydrochloric acid: The irritation of hydrochloric acid to silkworm eggs is due to the integrated action of three factors: concentration of hydrochloric acid (HCl), temperature, and duration of treatment, of which the concentration of HCl is the most important. Artificial hatching is less successful with a low-concentration acid treatment, but a high concentration will completely spoil the eggs. The concentration of HCl now being employed in acid treatment is 15 percent with a specific gravity of 1.075.

Temperature of HCl solution: Experience has proved that the effective range of the temperature of HCl solution is 45-47°C (113-116°F). The optimum is 46°C (115°F).

Duration of treatment: Under the conditions described above, five minutes is needed for acid treatment. This varies slightly with different races. For Chinese bivoltine eggs, five minutes is adequate, while Japanese bivoltines need five and a half minutes.

Acid treatment under normal or room temperature: In this method the acid is not preheated.

Optimum time for acid treatment: The optimum time for silkworm eggs to undergo acid treatment is 15-20 hours after they are laid and preserved at 24°C.

Concentration of the hydrochloric acid: For acid treatment under normal temperature, an optimum concentration of 21-22 percent (specific gravity 1.108-1.110) is comparatively safe and dependable.

Duration of treatment: The length of time needed for acid treatment varies greatly with different room (solution) temperatures. Slight variations also occur in case of early or late treatments. Table 8-3 indicates the duration range of acid treatment at different temperatures with an acid concentration of 21 percent (specific gravity 1.108).

8.4 Artificial Hatching by Acid Treatment after Cold Storage

In this method, the hibernating eggs first undergo cold storage, then are subjected to acid treatment. The actual procedure is as follows.

Right time for cold storage: Silkworm eggs are preserved at optimum temperature after being laid, 45-50 hours later the eggs begin to change colour from light yellow to brownish red. This is the right time for cold storage. In terms of embryonic development, this is the stage when the embryo becomes distinct with an enlarged protocephalic lobe, an expanded caudal lobe, and the formation of the mesoderm.

Table 8-3

Standard for the application of hydrochloric acid treatment at room temperature

Time for acid treatment		Duration of treatment (minutes)		
		Temp. of solution 24°C	Temp. of solution 27°C	Temp. of solution 29°C
Time lapse after eggs are laid and preserved at 24°C	10 hrs.	50-70	40-60	40
	15 hrs.	55-75	55-70	40-50
	20 hrs.	55-80	55-80	40-50
	25 hrs.	60-90	60-80	40-50

Cold storage temperature and time limit: The optimum temperature for cold storage is 5°C. The optimum duration for bivoltine eggs is 50-60 days. If acid treatment is applied forcibly sooner, hatching will not be uniform and the hatching rate will be low.

Appropriate interval for acid treatment after cold storage: When preserving eggs for acid treatment after cold storage, the temperature in the cold storage should be constant. On entering or leaving the cold storage, eggs must pass through a vestibule (approximately 16°C) for two hours. Acid treatment should not begin immediately after cold storage, but only after the eggs have been preserved at room temperature for an hour and a half to two hours.

Standard for acid treatment after cold storage: Usually, the degree of acid stimulation to eggs for acid treatment after cold storage, is stronger than that to eggs receiving common acid treatment. The standard adopted in production is:

Concentration of HCl: 19 percent (specific gravity 1.092)
Temperature of HCl solution: 47.8°C (118°F)
Duration of HCl treatment: Chinese race, 5-5.5 minutes
Japanese race, 6 minutes

Suspension of cold storage before acid treatment: Sometimes changes of plan necessitate that the eggs intended for acid treatment be released from the cold storage and come to a suspension (be changed into hibernating eggs). Silkworm races possessing stable hibernation characters and a deep diapause degree, have a longer safe period for suspension of cold storage. Nothing of importance occurs if the suspension takes place within a period of 20 days after the eggs are stored. But races with a rather unstable hibernation character, and a low diapause degree, the limit is 12 days in storage. In conclusion, the earlier suspension takes place, the better. If it takes place after the safe period, the phenomenon of continuous hatching will appear.

8.5 Manipulation of Acid Treatment

Preventing silkworm eggs from dropping: It is easy for the silkworm eggs produced by the flat-card method and Pasteur's method to drop off during acid treatment. Since formaldehyde has the property of coagulating the gummy substance on the silkworm egg surface, formalin solution is recommended to prevent egg-dropping during acid treatment. There are two methods:

(1) Adding formalin to the hydrochloric acid solution: An amount of formalin equivalent to 2 percent of the bulk of the objective dilute hydrochloric acid solution is added to the latter. The acid solution is heated and acid treatment commenced. Since formalin is very volatile, more formalin should be added during the application process.

(2) Spraying of formalin on silkworm eggs: A dilute solution of 1.5-2 percent formalin is sprayed on the silkworm eggs evenly, and left to dry before acid treatment. This method is more effective in preventing eggs from dropping during acid treatment at normal temperature.

Acid treatment: It is important to observe strictly all the criteria listed under acid treatment. During acid treatment, the silkworm eggs are expected to receive uniform heat treatment to achieve stable production. Eggs should be acid treated in the order that they are removed from cold storage.

Before putting the silkworm eggs into the acid solution, the temperature of the acid solution should be regulated to 0.3-0.5°C higher than the criterion temperature. After immersing the egg-card holder in the acid, it should be lightly pushed up and down to expel any air bubbles. The egg-card holder should be moved about in the acid container so that the eggs are heated uniformly (Figure 8-1).

Three temperature tests for the acid solution should be made during acid treatment and the results recorded. The first test is made one minute after immersion, and then every two minutes thereafter. If the temperature of the acid is lower than the criterion temperature, prolonging the time of treatment is preferable to raising the temperature of the acid. For example, for every 0.5°C per minute the temperature of the acid solution rises or falls. With respect to the objective temperature, treatment is shortened or lengthened respectively by five seconds.

Five seconds before reaching the acid treatment criterion time, the egg-card holder is lifted from the acid container and then deacidization is carried out. The consumption of dilute hydrochloric acid solution is about 25 cc for each egg-card and that of formalin is 1 kg for every 1 000 egg cards. The hydrochloric acid can be used repeatedly, but must be sieved, and in order to ensure the stimulating effect of the hydrochloric acid, its concentration should be adjusted.

Figure 8-1
Egg-card holder for acid treatment

Deacidization and drying: Deacidization means careful flushing of the eggs after acid treatment, in a basin of running water or in a flowing river, for less than one hour, until the acid has been thoroughly washed away. The requirements for deacidization are quickness and cleanliness. Also, it is important that the eggs should be prevented from dropping off. Although no destructive effect to the physiology of silkworm eggs has been detected, the shortest time for washing is recommended. Blue litmus paper may be used to determine whether the eggs have been completely deacidized or not.

After being thoroughly deacidized, the eggs should be dewatered in a dewatering centrifugal machine at a speed of 250 rpm for two minutes. Then, the egg-cards should be hung in a well- ventilated room. The egg-cards should air dry within 10 hours.

8.6 Hatching by Warm-Water Treatment

For diapause eggs of multivoltine silkworms, artificial hatching can be accomplished by warm-water immersion. This method originated in Guangdong Province.

When to apply treatment: Treatment should be conducted early in the morning, at about 05:00-07:00 hours, the day after the eggs are laid. When the weather is warm (27°C and above), the work should be done earlier, and later when the temperature is comparatively low (below 24°C).

Criteria for warm-water treatment: The temperature of the water is an important factor in the hatching of silkworm eggs. Low water temperature is less effective, but high water temperature may kill the eggs. The standard temperature for warm-water hatching depends on the preservation temperature after oviposition. In 1911, the sericulturist, Lu Chung-ming, proposed a formula for warm-water treatment:

$$A = \frac{B + C}{2}$$

A = Temperature of warm water (°F)
B = 180°F = Boiling point (212°F) - Freezing point (32°F)
C = Average preservation temperature after oviposition (°F)

In Celsius, it will be:

A = Temperature of warm water (°C)
B = (180-32) x 5/9 (°C)
C = Average preservation temperature after oviposition (°C)

From this formula, we may arrive at the following calculation:

Average preservation temperature after oviposition	Temperature of water
75°F	127.5°F (53.1°C)
80°F	130.1°F (54.5°C)
85°F	132.5°F (55.8°C)

Duration of immersion: The standard duration for water immersion is five seconds. However, when the water temperature falls, immersion can be lengthened. The rule is: for a reduction of 1°F of water temperature, immersion is increased one second, on the condition that the reduction of water temperature does not exceed the objective temperature by 2°F. Because immersion is so brief (five seconds), the work must be done in a very precise manner.

Disinfection of the egg surface: After warm-water treatment, the egg-cards should be hung until no more water drips from them. Disinfection of the egg surface may now begin. A 2.5 percent formalin solution should be prepared ahead of time, and the egg-cards immersed in the disinfectant for about 30 minutes.

Rinsing and drying: After disinfection, the silkworm eggs should be washed under running water for 20 minutes, in order to rid them of the chemical. They are then put into a dewatering centrifugal machine and hung to dry. If the humidity is high, an electric fan should be used to hasten drying. If the egg-cards cannot be dried in time, the physiological aspects of the eggs will be adversely affected and hatching will be not uniform.

CHAPTER 9

LOW-TEMPERATURE INHIBITION OF EMBRYONIC DEVELOPMENT OF SILKWORM EGGS

Low-temperature inhibition for artificial hatching and non-diapause multivoltine silkworm eggs is frequently employed as a temporary measure to defer the development of silkworm eggs.

9.1 Low-temperature Inhibition of Common-Acid-Treated Eggs

Low-temperature inhibition before common-acid-treatment: When common-acid-treatment cannot be carried out at a set time, it is possible to inhibit the development of the silkworm eggs temporarily with a low-temperature and to conduct acid treatment later on. However, rigid adherence to the following rules is necessary, or the procedure will fail.

(1) Duration of low-temperature inhibition: Silkworm eggs after being laid can be preserved either at 24°C for 18-22 hours, or at 26°C for 14-20 hours. It is essential to transfer the eggs to cold storage within this time limit.

(2) Requirements for low-temperature inhibition: Before they enter the main storage room, the silkworm eggs should be kept in the vestibule storage room at a temperature of 15°C for two hours. The temperature of the main storage room should be maintained at 5°C. Eggs should not be kept more than three days at such a low-temperature, and the less time the better.

(3) Silkworm eggs released from cold storage and subjected to acid treatment: Silkworm eggs released from the main cold storage room are left in the vestibule room for two hours and then at room temperature for another hour or two, prior to acid treatment. In this case, common acid treatment should be employed.

This method is adopted only when there is no alternative or when the amount of silkworm eggs is small, or when eggs that have been stored for two or three days must be acid treated at the same time.

Low-temperature inhibition after common-acid-treatment: This method is mainly utilized in tropical and sub-tropical areas. Embryos begin to develop after the eggs are treated with common acid. If their development is inhibited by low-temperature, the eggs can be taken out for incubation whenever needed.

The normal procedure is to preserve the eggs at 24-25°C for approximately 24 hours after acid treatment. By this time, the embryo has reached its period before the longest embryo stage (C_1). The eggs are chilled at 15°C for two hours in the cool vestibule room, and then transferred into the main cold storage at 5°C. Under this condition, the embryonic development can be inhibited for about 20 days.

9.2 Low-temperature Inhibition of Eggs Acid-Treated after Chilling

If the silkworm eggs that have received an acid treatment after being released from cold storage must be inhibited by low-temperature, they must be handled properly, or white dead eggs will result. The appearance of white dead eggs is caused by the duration and mode of chilling. If the eggs are inhibited by low-temperature 12-42 hours after acid treatment, a large amount of white dead eggs will most likely appear. However, if they are preserved at an intermediate temperature of 12.5°C for 12-18 hours before inhibition, the number of white dead eggs is remarkably reduced, possibly even to zero.

In production, low-temperature inhibition can be conducted as follows: the eggs are preserved at under 25-26°C for 36-42 hours after acid treatment. When the eggs develop to D embryonic stage, they should be put at an intermediate temperature of 12.5°C for 12-18 hours. The eggs should then be transferred to the main cold storage room at 5°C, where they can be inhibited for up to 20 days, though 15 days is preferable.

9.3 Low-temperature Inhibition for Non-Diapause Silkworm Eggs

In non-diapause silkworm eggs, embryonic development begins with oviposition. If date control of ant collection is required, punctuality in carrying out low-temperature inhibition is essential. The method is as follows: preservation is carried out at 25-27°C after oviposition for 30-40 hours. They should be kept at an intermediate temperature of 15°C in a cool vestibule room for 2-4 hours, then transferred to the main cold storage room at 5°C. The margin of safety is 15 days.

The resistance to low-temperature varies from one variety to another. For example, when comparing two non-diapause multivoltine varieties such as 115 Nan and Jiu Bai Hai, resistance to low-temperature of the latter is stronger than the former. The effective chilling period of Jiu Bai Hai is as long as 45 days.

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